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Cervicovaginal ureaplasmal infection alone is not predictive of preterm birth. Only a subpopulation of women infected in the lower genital tract are at risk for chorioamnion invasion and premature birth. The major goal of the proposed study is to identify microbiologic factors that predispose to and/or predict chorioamnion invasion and premature birth. This study will determine if the presence of bacterial vaginosis is a risk factor for ureaplasmal invasion of the chorioamnion. 1755 women have been enrolled to date. Vaginal cultures from 1497 of these women have been assessed for *Ureaplasma urealyticum* colonization and 564 have been assessed for Bacterial Vaginosis (BV). 731/149 or 48.8% are culturally positive for *U. urealyticum* and 101/564 or 18% of the gram stains are positive for BV. At delivery, the isolation rate of *U. urealyticum* from the vagina, placental tissues, amniotic fluid, and infant's noses is 60%, 10%, 14.8%, and 23% respectively. Gram stains for BV assessment at delivery is 16%. The persistence of *U. urealyticum* during pregnancy in this study sample is high, irrespective of delivery type (Cesarean section with intact membranes, Cesarean section with ruptured membranes, or vaginal delivery). This is important because of the asymptomatic nature of U. urealyticum and its detrimental effects in lowbirth pregnancies.

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FOREWORD

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INTRODUCTION:

Women are playing increasing roles in active duty military service and suffering unexplained adverse outcomes of pregnancy. In the Navy, up to 20% of enlisted women become pregnant each year and nearly 15% of these pregnancies suffer adverse effects. This percentage is in excess of that experienced in most other U.S. female populations and despite a number of preliminary investigations, the difference does not appear to be related to environmental exposures.

Some of the traditional factors associated with adverse outcomes of pregnancy such as limited access to prenatal care or poor nutritional status are not operative among naval personnel, nonetheless at least 10% of Navy live births are premature (<37 weeks gestation) or low birth weight. In addition, among pregnant, enlisted women, spontaneous abortions occur in 9.9%, 2.7% of pregnancies are ecotopic and 1.5% result in fetal death (0.7% early, 0.8% late). There is evidence that rates of spontaneous abortion among shipboard women may be higher than other military women and that shipboard women may frequently practice unprotected sexual intercourse (both while in port and at sea) and subsequently may acquire sexually transmitted pathogens. Bacterial infections of the lower genital tract may in part explain poor pregnancy outcomes.

Preterm birth complicates 8-10% of all pregnancies in the United States and is the leading cause of infant morbidity and mortality in the United States. We have shown that *Ureaplasma urealyticum* is the single most common microorganism isolated from the chorioamnion of women in spontaneous labor with intact membranes and in whom there are no chances for cervicovaginal contamination of the placenta (i.e. they delivered by cesarean section). Furthermore, ureaplasmal infection of the chorioamnion in the absence of other bacteria was associated with birth <37 weeks even after multifactorial analysis to adjust for labor and other obstetric and demographic factors that could confound the association. Infection was inversely related to gestational age and birth weight. Other related studies indicate that ureaplasmal infection is a significant cause of respiratory disease, meningitis and death in very low birth weight infants.

Cervicovaginal ureaplasmal infection alone is not predictive of preterm birth. It is clear that only a subpopulation infected in the lower genital tract are at risk for chorioamnion invasion and premature birth. In order to logically design therapeutic intervention trials, one must first be able to identify those at risk.

The major goal of the proposed study is to determine whether chorioamnion infection, in particular infection by *U. urealyticum*, is associated with adverse pregnancy outcome, specifically premature birth. The study population will include both active-duty military personnel, and dependents of active-duty military personnel.

The design of the study will also allow us to identify factors that may predict chorioamnion invasion and premature birth. Specifically we will determine if the presence of bacterial vaginosis (BV) is a risk factor for ureaplasmal invasion of the chorioamnion. Potential confounders of these data will be controlled through multivariate analyses. They include the presence of other sexually transmitted pathogens, in particular *Chlamydia trachomatis, Nisseria gonorrhoeae,* Group B streptococci, *Trichomonas vaginalis, Mycoplasma hominis,* and *Mycoplasma genitalium.*

In a 3-year enrollment period, approximately 11,000 women will be screened for *U. urealyticum* cervicovaginal colonization and BV. Of these women, about 1,272 will be followed at delivery and be re-cultured for BV and *U. urealyticum*. The population of 1,272 women will consist of: 672 cesarean sections (CS) with intact membranes; 300 CS with ruptured membranes and 300 vaginal deliveries. In addition to cultures for vaginal *U. urealyticum* and BV, the 1,272 women will also undergo culture of placental and amniotic fluid for aerobes, anaerobes, and ureaplasma/mycoplasma (placental tissue within one hour of delivery). Babies being born to these mothers will be assessed for gestational age, birth weight, and health status. Certain obstetric

conditions will be examined and results recorded on the questionnaire for all 1,272 study participants: whether or not PROM occurred (membrane rupture before the beginning of contractions regardless of gestational age); whether or not there was prolonged membrane rupture or labor (membrane rupture or labor for > 24 hr). Discharge diagnostic data from the delivery admissions of the other 9,828 (11,000 - 1,272 = 9,828) study participants will also be examined using an existing computerized hospital data base.

Upon first visit for prenatal care at Naval Medical Center, San Diego (NMCSD), both active-duty and dependent pregnant women, will be invited to participate in this study. After informed consent is granted, and in conjunction with other clinical specimen collection, study volunteers will be asked to permit collection of a vaginal swab and a 10cc blood specimen. A study nurse will complete the first four pages of the Mother's and Infant's Questionnaire for the volunteer, through an interview procedure and medical record review.

At delivery, the estimated 1,272 volunteers will undergo placental tissue cultures, amniotic fluid analyses and maternal sera analyses. It is anticipated that study serum will be collected along with other admission specimens. A 5- to 10-cc specimen of cord blood will also be taken after the umbilical cord is cut. This cord blood sample poses no inconvenience, danger or discomfort to the mother or infant and is routinely collected for clinical study. The infants' nasal secretions (at external nares) will be swabbed and cultured at birth. Other infant specimens (CSF and lower respiratory secretions) will only be collected if clinically indicated as determined by the patient's attending physicians.

The studies outlined in the present proposal will allow us to determine risk factors for chorioamnion infection and association with adverse pregnancy outcome (including prematurity and spontaneous abortion). These studies will also provide a comprehensive analysis of the incidence of sexually transmitted pathogens in pregnant women in the Navy. These data not only will facilitate the future design of rational treatment strategies but they will also allow comparison of different populations of women in military service and potential environmental and sociological effects on pregnancy outcome. Considering the fact that we have recently shown *U. urealyticum* chorioamnion infection to be a significant risk factor for postpartum endometritis, the studies in the present proposal should also provide information useful for reducing hospital costs and morbidity associated with this delivery complication.

To accomplish this study, a study nurse coordinator, a clinical microbiologist and an after hours on-call technician have been hired specifically to screen and enroll patients, process specimens, examine cultures and identify all microorganisms, and interpret data. These UAB employees live in the San Diego area and are enrolling the women (active duty and dependent wives) being seen at the OB/GYN clinic of the Naval Medical Center, San Diego as their patient population.

BODY:

Experimental methods to be used in this study are identical to those detailed in the original proposal.

As of September 30, 1997, 1755 patients have been consented and enrolled into the study. Enrollment is summarized in the following Table.

Table 1

Chorioamnion Infection Study: enrollment statistics from June '96 to Sept'97					
	# Res in clinic	# seen by UAB	# enrolled by UAB	% seen by UAB	Enrollment rate
June '96 (6/10-28)	271	96	64	35%	67%
July '96	454	98	65	22%	66%
Aug '96 (didn't enroll entire month)	217	73	53	34%	73%
Sept '96	309	69	47	22%	68%
Oct '96	460	125	95	27%	76%
Nov '96	241	79	61	33%	77%
Dec '96	301	62	55	21%	89%
Jan '97	448	132	119	29%	90%
Feb '97	457	129	103	28%	80%
*March '97 (mar 24 self-swab)	387	149	129	39%	87%
April '97	437	285	197	65%	69%
May '97	384	232	159	60%	69%
June '97	474	280	175	59%	63%
July '97	472	263	173	56%	66%
August '97	337	186	117	55%	63%
Sept '97	330	210	143	64%	68%
Total:	5979	2468	1755		

Although the number is lower than what we previously predicted, our numbers continue to improve. As we proposed in the 1996 Progress Report, a pilot study to compare self-swabbing and physician obtained vaginal specimens was approved and conducted. The results of this pilot study were favorable and are shown below.

Table 2
Results from Self-swab Pilot

	Self- swabbed	Physician obtained
Negative	22	24
Ureaplasma urealyticum only	27	25
Mycoplasma species only	1	1
Ureaplasma urealyticum and mycoplasma species	3	4
Overgrown with Bacteria	1	0
Overgrown with Yeast	1	1
Bacterial Vaginosis	9 +	10+
Trichomonas	53 -	53 -

By converting to this method of collection in our protocol, our enrollment numbers have increased as indicated in figure 1. Self-swabbing began on March 24, 1997. A revised copy of the consent form can be found in Appendix 1.

Demographics of the enrollees (those attending the Naval Medical Center San Diego Obstetrics clinic for their first pre-natal visit) to date are demonstrated below:

Age Range	15-43	(51 < 18 years of age)	
Race	White	887/1674	53%
	Black	227/1674	13.6%
	Asian/PI	246/1674	14.7%
	Eskimo/Aleut./American Indian	12/1674	0.7%
	Spanish/Hispanic	227/1674	13.6%
	Other	66/1674	3.9%
	Multiple	9/1674	0.5%
Military Status	Navy	340/1490	22.8%
	Marine	13/1490	0.8%
	Army	1/1490	<0.1%
	Public Health	1/1490	<0.1%
	Reserve	11/1490	0.7%
	National Guard	1/1490	<0.1%
	Retired Military	16/1490	1.1%
	Civilian	1107/1490	74.3%
Medical Care	Within previous year	916/1646	55.7%
	1-2 years	488/1646	29.6%
	3-5 years	132/1646	8%
	>5 years	74/1646	4.5%
	Never	36/1646	2.2%

Patient questionnaires in the scantron format have simplified data entry. Other data obtained and ready for analysis are those factors which could contribute to pre-term labor and include general health questions, exposure to smoke, consumption of alcohol, use of drugs, dental hygiene, previously diagnosed sexually transmitted diseases, and previous problem pregnancies. An example of this questionnaire and other data collection forms can be found in Appendix 2.

Of the 1755 women screened at their initial pre-natal visit, 564 slides have be accessed for Bacterial Vaginosis by the Nugent Gram stain method. 101/564 or 18% are positive for BV (a score of $\geq$ 7)while 45/564 or 8% are considered to be intermediate (scoring 4-6). 74% or 417/564 are negative for BV (score $\leq$ 3). Trichimonas has been isolated from 16 patients (0.9%). Chart reviews are necessary to capture the Chlamydia results. Of 132 charts reviewed, 8/132 or 6% of the study population are positive for Chlamydia. The hospital reports an incidence of 8-10% from their general Obstetric and Gynecology population. Ureaplasma urealyticum and mycoplasma species incidence is found in Table 3.

Table 3
Ureaplasma urealyticum and Mycoplasma species
Pre-natal Screens
N=1498

Negative	601
Ureaplasma urealyticum only	731
Mycoplasma species only	15
Ureaplasma urealyticum and mycoplasma species	140
Overgrown with Bacteria	3
Overgrown with Yeast	8

As of October 1, 1997, 662 women enrolled in the UAB study have delivered. The outcome of those deliveries are summarized in figure 2. By following these outcomes, we have a better understanding of the population which we are studying. Only 12% of the deliveries are by cesarean section. Of those, 6.3% have ruptured membranes while 5.6% have membranes intact. To date, 163 women have met study criteria (all cesarean section with membranes intact, randomly selected cesarean section with ruptured membranes, randomly selected vaginal deliveries) and have completed the study i.e. vaginal swabs prior to delivery for BV-acessment and Ureaplasma urealyticum colonization, placenta and amniotic fluid for culture of aerobes, anaerobes and ureaplasmas and mycoplasmas, and infant nasal cultures for ureaplasma and mycoplasma colonization. Cultures for aerobes and anaerobes are processed on sight within an hour of delivery. Cultures for ureaplasmas and mycoplasmas are frozen at -70^0 C and are batched and sent to the UAB reference laboratory on dry ice monthly. Vaginal cultures collected just prior to delivery were positive for Uu only in 73/121 (60%) of the patients. One patient had mycoplasma species only (0.8%) and there were 5 patients that were culturally positive for both Uu and mycoplasma species (4%). BV acessment has been performed on 91 of the 164 slides. 15/91 (16%) were graded as positive, 20/91 (22%) graded intermediate and 56/91 (62%) were considered to be negative for BV. Uu was isolated from the placental tissues in pure culture in 11/126 (8.7%) of the patients and mixed with mycoplasma species in 2 patients (1.6%). The membrane specimen is actually collected between the chorion and the amnion and 11/126 (8.7%) were positive for Uu only. 4/27 (14.8%) of the amniotic fluids were positive for Uu only and 28/121 (23%) of the infant's nasal passages were colonized with Uu only. Culture results are found in the tables below.

Table 4a. C-section intact membranes
Ureaplasma and Mycoplasma Results

	Vaginal	Placental TX	Membrane	AF	Nasal
Negative	9	23	23	28	22
Ureaplasma urealyticum only	10				
Mycoplasma sp. Only					
Ureaplasma urealyticum and Mycoplasma sp.	1				
Overgrown with Bacteria	1				
Overgrown with yeast					

Table 4b. C-section ruptered membranes
Ureaplasma and Mycoplasma Results

	Vaginal	Placental TX	Membrane	AF	Nasal
Negative	3	16	17	1	13
Ureaplasma urealyticum only	16	4	3	4	6
Mycoplasma sp. Only					
Ureaplasma urealyticum and Mycoplasma sp.	1				
Overgrown with Bacteria	1				
Overgrown with yeast					

Table 4c. C-section intact membranes
Ureaplasma and Mycoplasma Results

	Vaginal	Placental TX	Membrane	AF	Nasal
Negative	9	23	23	28	22
Ureaplasma urealyticum only	10				
Mycoplasma sp. Only					
Ureaplasma urealyticum and Mycoplasma sp.	1				
Overgrown with Bacteria	1				
Overgrown with yeast					

Culture negative results for aerobes and anaerobes were detected in 85/163 patients' specimens or 52% .

Table 5a. C-section intact membranes
Aerobe and Anaerobe Results
(N=37)

	Placental TX	Membrane	AF
Alpha-hemolytic streptococcus	0	0	0
Group D Streptococcus, non enterococcus	0	0	0
Group B Streptococcus	0	0	0
Gamma Streptococcus species	0	0	0
Corynebacterium species (Dipheriods)	0	1	2
Gardnerella vaginalis	2	2	1
Micrococcus species	0	0	0
Staphylococcus aureus	0	0	1
Coagulase Negative Staphylococcus species	1	0	1
Coagulase Negative Staphylococcus not saphrolyticus	0	1	5
Propionibacterium avidum	0	0	1
Propionibacterium acnes	0	0	0
Propionibacterium granulosum	0	0	0
Propionibacterium species	0	0	6
Veillonella species	0	0	0
Disulfomona species	0	0	0
Actinomyces meyeri	0	0	0
Bacteroides vulgatus	0	0	0
Peptostreptococcus asaccharolyticus	0	0	0
Peptostreptococcus magnus	0	0	0
Eubacterium lentum	0	0	0
Lactobacillus species	0	0	0
Lactobacillus jensenii	0	0	0
Lactobacillus fermentum	0	0	0
Mobiluncus species	0	0	0
Citrobacter species	0	0	1
Escherichia coli	0	0	0
Bacillus species	0	0	0
Bactéroides uniformis	0	0	0

Table 5a. (continued) C-section intact membranes
Aerobe and Anaerobe Results (N=37)

	Placental TX	Membrane	AF
Peptostreptococcus prevotii	0	0	0
Peptostreptococcus anaerobius	0	0	0
Peptostreptococcus species	0	0	0
Beta-hemolytic Streptococcus species not ABCDFG	0	0	0
Candida albicans	0	0	0
Trichomonas vaginalis	0	0	0

Table 5b. C-section ruptured membranes
Aerobe and Anaerobe Result (N=31)

	Placental TX	Membrane	AF
Alpha-hemolytic streptococcus	2	0	0
Group D Streptococcus, non enterococcus	0	0	0
Group B Streptococcus	0	0	0
Gamma Streptococcus species	0	0	0
Corynebacterium species (Dipheriods)	1	0	0
Gardnerella vaginalis	2	0	1
Micrococcus species	0	1	0
Staphylococcus aureus	0	0	0
Coagulase Negative Staphylococcus species	0	0	0
Coagulase Negative Staphylococcus not saphrolyticus	1	1	0
Propionibacterium avidum	1	0	0
Propionibacterium acnes	2	0	1
Propionibacterium granulosum	0	0	0
Propionibacterium species	0	0	0
Veillonella species	0	0	0
Disulfomona species	0	0	0
Actinomyces meyeri	0	0	0
Bacteroides vulgatus	0	0	0
Peptostreptococcus asaccharolyticus	1	1	0
Peptostreptococcus magnus	0	0	0
Eubacterium lentum	1	0	0
Lactobacillus species	0	0	1
Lactobacillus jensenii	0	0	0
Lactobacillus fermentum	0	0	0
Mobiluncus species	0	0	0
Citrobacter species	0	0	0
Escherichia coli	0	0	0
Bacillus species	0	0	0
Bacteroides uniformis	0	0	0
Peptostreptococcus prevotii	0	0	0
Peptostreptococcus anaerobius	0	0	0
Peptostreptococcus species	0	0	0
Beta-hemolytic Streptococcus species not ABCDFG	0	0	0
Candida albicans	0	0	0
Trichomonas vaginalis	0	0	0

Table 5c.
Vaginal Deliveries
Aerobe and Anaerobe Results (N=95)

	Placental TX	Membrane
Alpha-hemolytic streptococcus	5	2
Group D Streptococcus, non enterococcus	3	3
Group B Streptococcus	4	1
Gamma Streptococcus species	1	3
Corynebacterium species (Dipheriods)	4	1
Gardnerella vaginalis	7	3
Micrococcus species	2	1
Staphylococcus aureus	1	0
Coagulase Negative Staphylococcus species	1	0
Coagulase Negative Staphylococcus not saphrolyticus	6	4
Propionibacterium avidum	0	0
Propionibacterium acnes	2	1
Propionibacterium granulosum	1	0
Propionibacterium species	2	0
Veillonella species	1	1
Disulfomona species	0	0
Actinomyces meyeri	1	0
Bacteroides vulgatus	1	0
Peptostreptococcus asaccharolyticus	1	0
Peptostreptococcus magnus	1	0
Eubacterium lentum	0	0
Lactobacillus species	5	4
Lactobacillus jensenii	1	0
Lactobacillus fermentum	1	0
Mobiluncus species	0	0
Citrobacter species	0	1
Escherichia coli	2	2
Bacillus species	0	1
Bactéroides uniformis	1	0
Peptostreptococcus prevotii	0	1
Peptostreptococcus anaerobius	1	0
Peptostreptococcus species	1	0
Beta-hemolytic Streptococcus species not ABCDFG	0	1
Candida albicans	1	0
Trichomonas vaginalis	0	0

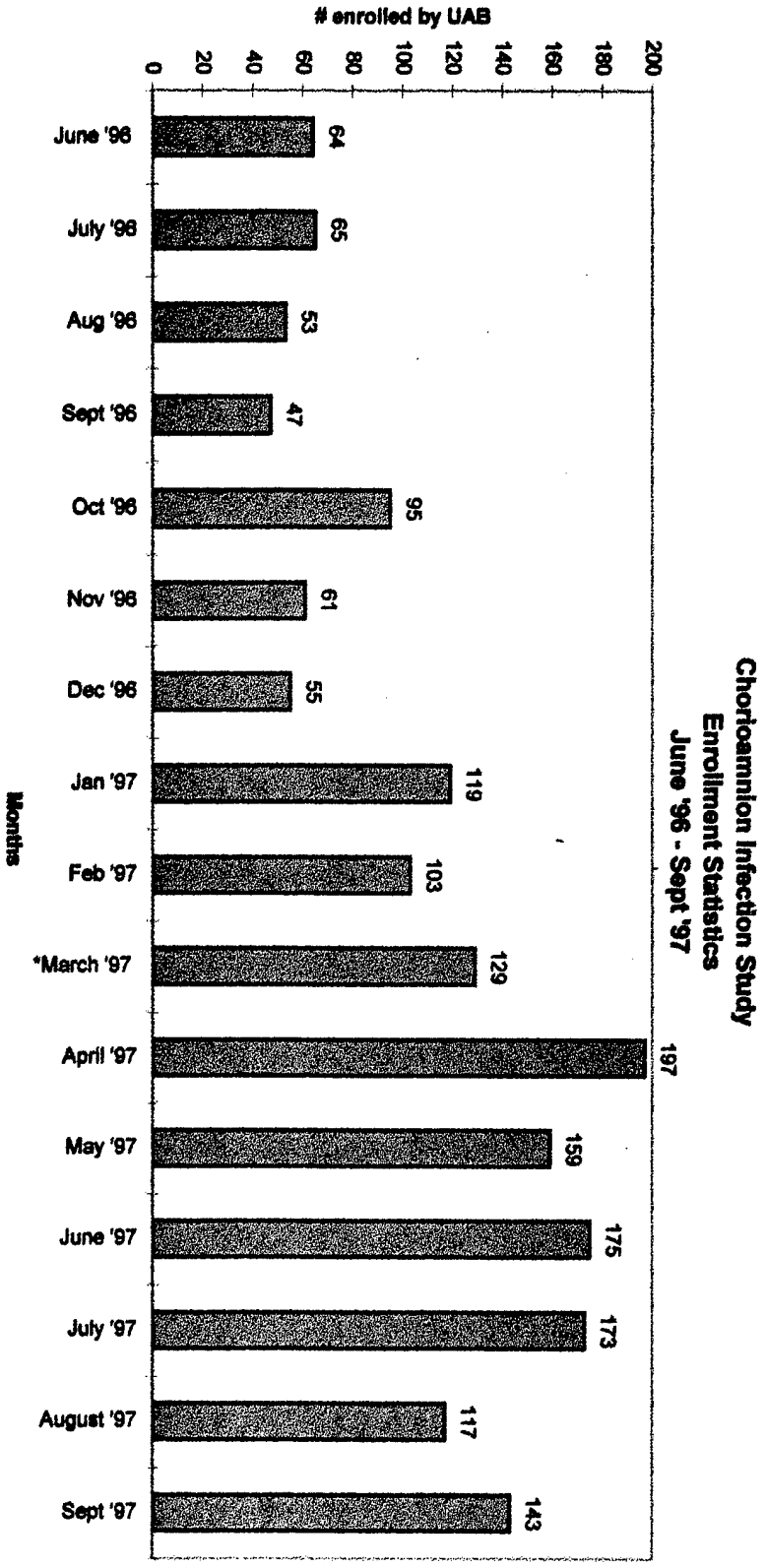
To date, 34 study infants have been followed-up with additional cultures after admittance to the Neonatal Intensive Care Unit. Cultures collected were one of the following: endotracheal aspirate, nasopharyngeal swab (NP), blood, and cerebrospinal fluid (CSF). All NP, blood, and CSF cultures were negative. Endotrachael aspirates were collected on only those infants which had been intubated. Four were positive for *Ureaplasma urealyticum.*

A small subset of these patients were recently analyzed for a medical student presentation. This project was to compare polymerase chain reaction (PCR) versus culture in the detection capability for *Ureaplasma urealyticum.* A copy of this presentation is in Appendix 3.

CONCLUSION:

The population is well defined and enrollment is increasing. However, at delivery there have been only 36 cesarean sections with intact membranes to date and only nine of those were preterm birth. The persistence of *U. urealyticum* during pregnancy in this study sample is high, irrespective of delivery type (Cesarean section with intact membranes, Cesarean section with ruptured membranes, or vaginal delivery). This is important because of the asymptomatic nature of *U. urealyticum* and its detrimental effects in lowbirth pregnancies.

Our personnel in San Diego has increased to 1 full-time study nurse, 1 clinical microbiologist, 1 Medical Technologist, 1 patient enroller, 1 on-call duty person, and a data entry person. The increased staff (over and above that in the original budget proposal) was needed to provide the services necessary to meet our study goals. The systems that are in place including clinic enrollment, labor and delivery notification, and nursery participation are working extremely well due to the superior staff that is coordinating these efforts. Examples of study updates and information used at in-service training are included in Appendix 4.


Chorioamnion Infection Study
Enrollment Statistics
June '96 - Sept '97
enrolled by UAB
0
20
40
60
80
100
120
140
160
180
200
Months
June '96
64
July '96
65
Aug '96
53
Sept '96
47
Oct '96
95
Nov '96
61
Dec '96
55
Jan '97
119
Feb '97
103
*March '97
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April '97
197
May '97
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June '97
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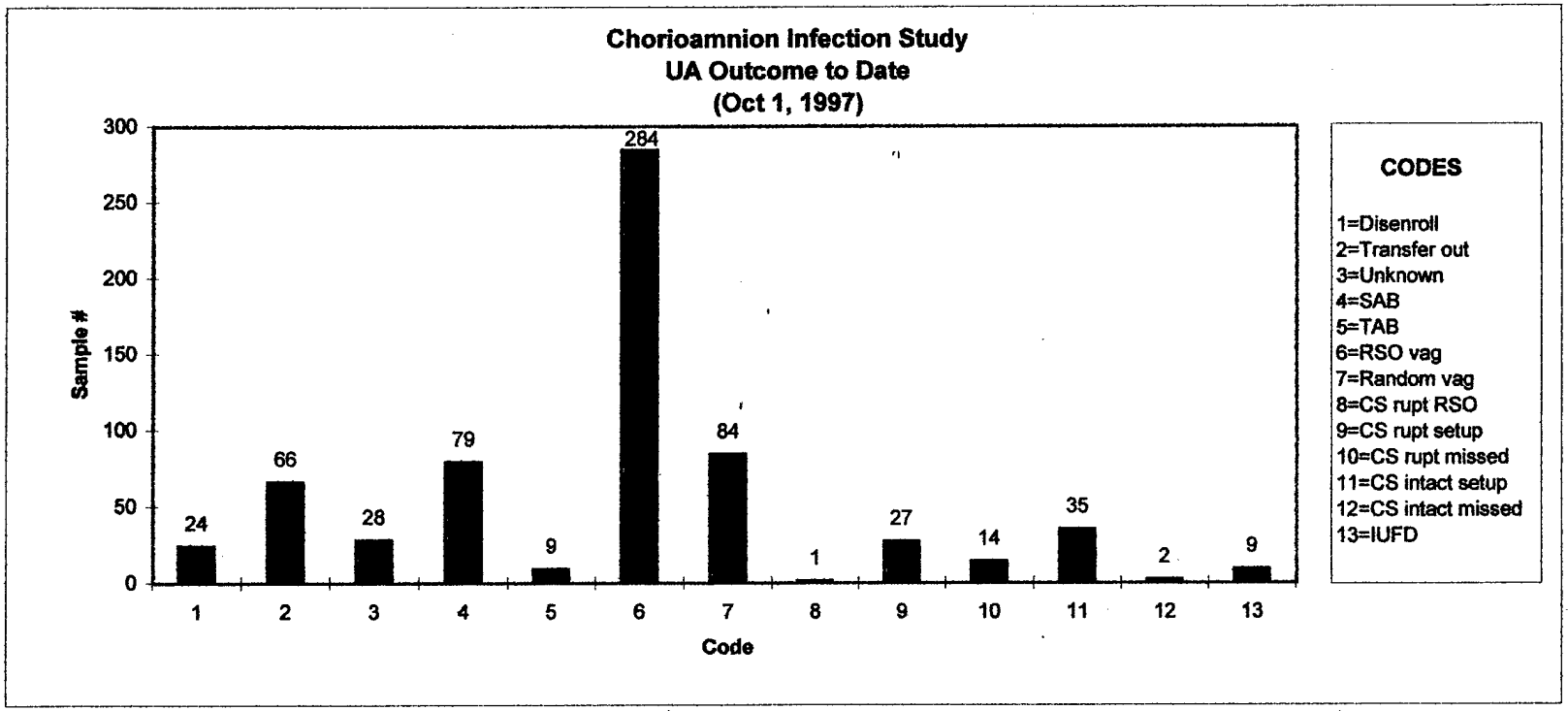
July '97
173
August '97
117
Sept '97
143

Figure 1



Outcomes

% Disenroll =	3.60%
% Transer out =	10.0%
% Unknown =	4.20%
% SAB =	12.0%
% TAB* =	1.40% (self-reported)
% Vag deliveries =	56.0%
% C sec =	12.0%
% IUFD =	1.40%

Figure 2

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APPENDIX 1

THE UNIVERSITY OF
ALABAMA AT BIRMINGHAM

Department of Microbiology
Office of the Chairman

October 13, 1997

Elizabeth Austin
United States Army Medical Research and Materiel Command
Research Area Director VI
Attention: MCMR-AAA-B
Fort Detrick
Frederick, MD 21702-5014

Dear Ms. Austin:

I am writing this letter to inform you of my new career move. I have recently resigned as the Chairman of the Department of Microbiology at the University of Alabama at Birmingham. On November 1, 1997, I will become Vice President of infectious diseases discovery research and clinical investigation worldwide for Eli Lilly and Company headquartered in Indianapolis, Indiana. However, I will retain my appointment as tenured professor in the Department of Microbiology at UAB hence the status of my clinical laboratory will not change, but will remain intact with all original personnel. I will continue to regularly monitor the study both at UAB and in San Diego. I will return to UAB monthly to review my on-going studies and clinical projects. My staff will be able to easily reach me by phone, FAX and e-mail.

I notified Dan Signore by phone who stated that he did not feel that my career change would be a concern regarding the ongoing studies. He indicated that since he also has recently changed assignments that I should include a letter with the progress report stating my change in title

I appreciate the opportunity that we have had in this joint venture and look forward to continuing with this and possibly future projects.

Sincerely,

Gail H. Cassell

Gail H. Cassell, Ph.D.
Charles H. McCauley, Professor and Chair

The University of Alabama at Birmingham
276 Bevill Biomedical Research Building • 845 19th Street South
Birmingham, Alabama 35294-2170 • (205) 934-9339 • FAX (205) 934-9256

NAVAL MEDICAL CENTER
SAN DIEGO, CALIFORNIA 92134-5000

CONSENT *BY A SUBJECT* FOR VOLUNTARY PARTICIPATION IN A CLINICAL INVESTIGATION (RESEARCH) STUDY

1. I (we), _____, have been asked to voluntarily participate in a research project entitled, "Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome among Active-Duty Military Women and Dependent Women," being conducted at the Naval Medical Center, San Diego (NMCSD) and in collaboration with investigators from the University of Alabama at Birmingham (UAB) and the Naval Health Research Center (NHRC), San Diego.

2. The purpose of this research project is designed to determine if pregnant women who have a common bacteria called Ureaplasma urealyticum (Uu) or other bacteria in their vaginas [bacterial vaginosis (BV)] are more at risk for premature delivery.

3. I (we) understand that my participation in this research project will be for a period of approximately nine months. I understand that the father of my baby will sign this consent form (if he is available) as requested by the U.S. Army Medical Research and Materiel Command (USAMRMC) according to the Office Protection of Research Rights, Protection of Human Subjects Code of Federal Regulations 45 CFR 46, paragraph 207(b).

4. The procedures for this project include the following:

a. On my first visit for prenatal care at NMCSD, I will be asked to obtain my own vaginal swabs. I will be given written instructions for the self-swabbing technique. I will also be asked to permit a 10cc blood specimen (2 teaspoons). I will complete Part 1 (four pages) of the Mother's and Infant's Questionnaire. The questions will be similar to ones my physician would normally ask were I not to volunteer for this study. The first four pages of the questionnaire will take approximately 15 minutes of my time to complete. Part 2 of the questionnaire which is completed by those women who meet the criteria listed in 4a will be completed by a medical record review done by the study nurse. The questions regarding drug use and sexually transmitted diseases are of a sensitive and personal nature but could have relevance in predicting premature labor or other adverse pregnancy outcomes.

Subject's Initials: _____

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I (we) understand that only women who meet certain criteria will participate in the remainder of the study (study sample will be 1,272 women). These criteria are: delivery by cesarean section with membranes intact at the time of delivery (bag of waters has not broken); selection from a random sample of women who deliver by cesarean section with ruptured membranes at the time of delivery (bag of waters has broken); or selection from a random sample of women who deliver vaginally. At delivery, a further 10cc blood specimen will be collected along with other admission specimens. A 5 to 10cc specimen of cord blood will also be taken after the umbilical cord is cut. This cord blood sample poses no inconvenience, danger or discomfort to me or to my infant and is routinely collected for clinical study. I (we) understand that amniotic fluid may be collected at the time of delivery for research purposes only and that additional vaginal swabs will be performed. I (we) understand that my placenta will be studied with a number of routine cultures performed. I (we) understand that the specimens described in section 4d may be collected from my (our) baby.

b. I (we) understand that the procedures for collecting specimens in this study will not post any increased risk to me or my (our) baby. I (we) understand that the study procedures will not interfere with or delay the standard of care for me and for my (our) infant.

c. I (we) understand that researchers will also study the specimens mentioned above for evidence for chlamydia, yeast, gonorrhea, Group B streptococcus and syphilis. Even if I (we) were not volunteering to participate in this research study, physicians would normally conduct these tests.

d. I (we) understand that my (our) infant will have his/her nose cultured by placing a soft swab into his/her nostril for approximately 3 seconds. I (we) also understand that if, at any time, my (our) infant's clinical condition requires that he/she be put on a breathing machine, respiratory secretions will be studied for the bacteria Uu. Also, if my (our) infant becomes sick and a spinal tap (collection of cerebral spinal fluid from the spinal canal) is required, excess fluid will be studied for this bacteria. I (we) understand that cerebral spinal fluid will not be collected routinely on all infants, only those who require a spinal tap. Although the specimens collected on my (our) baby are invasive, I (we) will be counseled by trained personnel on any possible risks and informed consent will be obtained for the spinal tap (if a spinal tap is necessary).

5. All women seeking prenatal care at NMCSD will be invited to participate in the study. Of these, comprehensive placental cultures will be performed on 672 women who have cesarean sections with intact membranes, 300 women who have cesarean

sections with ruptured membranes and 300 women who deliver vaginally.

6. The risks or discomforts which are possibly related to my participation in this study are as follows:

I may feel some mild pain at the site where blood is taken. Sometimes a bruise may occur. Rarely, a small clot may develop or local swelling and bleeding from the puncture site may be seen. The vaginal cultures will be performed with soft swabs and will be no more uncomfortable than placing a tampon. I will not feel any discomfort from specimen obtained from my placenta, amniotic fluid or umbilical cord.

7. I understand that my participation in this research project may or may not be of direct benefit to me personally or to my (our) infant. However, if I am found to be infected with a known pathogen (bacterium or virus), I will benefit by appropriate treatment. This treatment in turn may provide a direct benefit to my (our) infant, as early detection of and treatment for pathogens in the mothers result in improved quality of the gestation and birth periods for the infant. After birth, my (our) infant will continue to receive direct benefits. The neonatologist will have immediate knowledge of whether my (our) infant tests positive for Uu and will be able to administer the necessary treatment in a timely fashion. The results of this study may aid the investigator in gaining important knowledge about the prevention of poor outcomes of pregnancy (i.e., preterm births) among military active-duty and dependent women and aid in the future medical evaluation and treatment of other patients and their infants.

8. The alternate procedures or course of treatment, should I (we) decide not to participate in this research study, has been explained to me as follows: I (we) understand that I (we) do not have to participate in this research study and can receive the standard medical care for prenatal visits and delivery if I (we) do not participate.

9. In all publications and presentations resulting from this research study, information about me or my participation in this project will be kept in the strictest confidence and will not be released in any form identifiable to me personally. However, I (we) realize that authorized personnel from the Navy Medical Department and from the Food and Drug Administration (FDA), where applicable, may have access to my research file in order to verify that my rights have been adequately protected.

a. The information provided in this study will be analyzed by UAB, NHRC and NMCSD. The study documents and data files will be maintained by the UAB and NHRC where they will be used to

Subject's Initials: _____

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study trends among pregnant military women. Medical research information will be used for analysis and reports by UAB and Departments of the Navy and Defense and other U.S. Government agencies. Use of the information may be granted to non-Government agencies or individuals by the Navy Surgeon General following the provisions of the Freedom of Information Act or contracts and agreements. I (we) voluntarily agree to its disclosure to the agencies or individuals identified above.

b. All responses will be held in confidence by UAB, NMRC and NMCSD. All samples sent to UAB will be coded with the last four digits of the sponsor's social security number and a study number. Information that I (we) provide will be considered only when statistically summarized with the responses of others and will not be attributed to any single individual in publications.

c. I (we) understand that it is the policy of USAMRMC that data sheets are to be completed on all volunteers participating in research for entry into the Command's Volunteer Registry Data Base. The information to be entered into this confidential data base includes my name, address, social security number, study name and dates. The intent of the data base is two-fold: first, to readily answer questions concerning my participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure I am adequately warned of risks and to provide me (us) with new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years.

10. If I (we) suffer any injury directly related to my participation in this research study, immediate medical attention is available at the Naval Medical Center, San Diego or at another closer military medical treatment facility, if applicable. I (we) understand that although no financial compensation is available, any injury resulting from my (our) participation in this study will be evaluated and treated in keeping with the benefits or care to which I am (we are) entitled under applicable Navy, other Department of Defense, and other state or Federal regulations.

11. If I have any questions regarding this research study, I may contact Dr. Gray, (619) 553-9967. If I (we) have any questions about my (our) rights as individuals while participating in the research study at the Naval Medical Center, San Diego, I (we) my contact CDR Dean Gubler, MC, USN, Chairman, Committee for the Protection of Human Subjects at (619) 532-8125, or CDR Dennis Reeves, MSC, USN, Department Head, Clinical Investigation Department at (619) 532-8127. If I believe that I have been injured as a result of my participation in this research study, I may contact CDR Lynn McNees, JAGC, USN, Naval Medical Center, San Diego, Legal Department, at (619) 532-6475.

Subject's Initials: _____

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12. I (we) understand that my (our) participation in this project is entirely voluntary and that my (our) decision not to participate will involve no penalty or loss of benefits to which I am (we are) entitled under applicable regulations. If I choose to participate, I am free to ask questions or to withdraw from the study at any time. If I should decide to withdraw from the research project, I (we)will notify **Dr. Gray (619) 553-9967** to ensure my (our) timely removal from the study. My (our) withdrawal will involve no prejudice to my (our) future health care or any loss of rights or benefits to which I am (we are) otherwise entitled. Any new significant finding developed during the course of this study which might affect my (our) willingness to continue participation will be communicated to me (us).

13. The investigator may terminate my (our) participation in this study for the following reasons: The investigator or I (we) may terminate my (our) participation in this study at any time. Possible reasons for ending my (our) participation may include my (our) failure to cooperate or a new finding that continuing the study increases my risk of illness.

14. I (we) understand that I am (we are) making a decision whether or not to participate in the research project described in the preceding sections subject to the conditions of participation described above. My (our) signature indicates that I (we) have decided to participate, having read and understood the information presented above and having been given the opportunity to ask any questions that I (we) might have about the research study or my participation in the study. Further, my (our) signature indicates that I (we) have been provided with a copy of this consent document and a copy of a document entitled, "California Experimental Subject's Bill of Rights."

SIGNATURES AND **DATE** SIGNED: **PRINTED** OR **TYPED IDENTIFICATION:**

Patient / Subject (Date)	Name / Status / Sponsor's SSN
Witness (Date)	Name / Grade or Rank / SSN
Researcher/Investigator(Date)	Name / Grade or Rank / SSN
Father of Infant (Date)	Name / Grade or Rank / SSN

CPHS APPROVED
DATE 9/10/97
INIT
NAVAL MEDICAL CENTER SAN DIEGO

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CALIFORNIA EXPERIMENTAL SUBJECTS BILL OF RIGHTS

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment or who is requested to consent on behalf of another has the right to:

1. Be informed of the nature and purpose of the experiment;

2. Be given an explanation of the procedures to be followed in the medical experiment and any drug or device to be used;

3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment;

4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable;

5. Be given a disclosure of appropriate alternative procedures, drugs, or devices that might be advantageous to the subject and their relative risks and benefits;

6. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if any complications should arise;

7. Be given an opportunity to ask any questions concerning the experiment or the procedures involved;

8. Be instructed that the consent to participate in the medical experiment may be withdrawn at any time, and the subject may discontinue participation in the medical experiment without prejudice;

9. Be given a copy of a signed and dated written consent form when one is required;

10. Be given the opportunity to decide to consent or not consent to medical experiment without intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision; and

11. Be assured that the subject's confidentiality will be preserved and his/her name will not be released without his/her permission.

Any questions regarding this research study should be directed to the principal investigator or associate investigators. Information is available from the **Chairman, Committee for the Protection of Human Subjects**, established for the protection of volunteers in research projects at this facility by calling **(619) 532-8125** or writing the Chairman, Committee for the Protection of Human Subjects at Naval Medical Center, Clinical Investigation Department (Code AVA), San Diego, CA 92134-5000.

PRIVACY ACT STATEMENT

1. Authority. 5 USC 301

2. Purpose. Medical research information will be collected to enhance basic medical knowledge or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury, or functional impairment.

3. Use. Medical research information will be used for statistical analysis and reports by the Department of the Navy, the Department of Defense, and other U.S. Government agencies, provided this use is compatible with the purpose for which the information was collected. Use of the information may be granted to non-Government agencies or individuals by the Chief, Bureau of Medicine and Surgery in accordance with the provisions of the Freedom of Information Act.

4. Disclosure. I understand that all information contained in this Consent Statement or derived from the medical research study described herein will be retained permanently at Naval Medical Center San Diego and salient portions thereof may be entered into my health record. I voluntarily agree to its disclosure to agencies or individuals identified in the preceding paragraph. I have been informed that failure to agree to such disclosure may negate the purposes for which the research study was conducted.

SIGNATURES AND DATE SIGNED: **PRINTED OR TYPED IDENTIFICATION:**

Patient / Subject (Date) (if Applicable)	Name / Status / Sponsor's SSN
Parent / Guardian (Date) (if Applicable)	Name / Status / SSN
Witness (Date)	Name / Grade or Rank / SSN

APPENDIX 2

This questionnaire will help us provide the best medical care to mothers and their infants. **WE NEED YOUR HELP.** Note that this information will remain confidential and is obtained for investigational purposes only.

IMPORTANT INSTRUCTIONS

* **USE NO. 2 PENCIL ONLY.**
* **Do NOT use ink, ballpoint, or felt tip pens.**
* **Erase cleanly and completely any changes you make.**
* **Make black marks that fill the circle.**
* **Do NOT make any stray marks on the form.**

SECTION A: Maternal Demographics

What is today's date?

MO. DAY YR.

What is your SPONSOR'S Social Security Number?

SOCIAL SECURITY NUMBER

What is YOUR birthdate?

MO. DAY YR.

What is YOUR current age?

YRS

Study ID #

5. What are YOUR initials?

First
Middle
Last

6. What is YOUR home address? _____

Street

_____

City State Zip Code

7. What are YOUR phone numbers?

Work Home

8. Did the father of your baby sign the consent form?

- ○ Yes
- ○ No

9. What is YOUR CURRENT marital status?

- ○ Never married
- ○ Married
- ○ Separated
- ○ Divorced
- ○ Widowed
- ○ Other

10. Which is the BEST descriptor of YOUR ethnic/ racial background?

- ○ White/Caucasian
- ○ Black/African-American
- ○ Asian or Pacific Islander
- ○ Eskimo, Aleut, or American Indian
- ○ Spanish/Hispanic
- ○ Other (specify)_____

11. What is the HIGHEST level of education YOU have completed?

- ○ 11th grade or less
- ○ 12th grade or GED
- ○ 1-2 years of college/trade school
- ○ 3 or more years of college/trade school
- ○ 1 year or more of graduate or professional school

Please complete questions 12 and 13 regarding your and the baby's father's occupation.

12. What is the BEST description of your and the baby's father's CURRENT military or occupational status? (Select only ONE for each of you.)

YOU	FATHER	
○	○	Active Duty - Navy
○	○	Active Duty - Marine Corps
○	○	Active Duty - Army
○	○	Active Duty - Air Force
○	○	Active Duty-Public Health Service
○	○	Reservist
○	○	National Guard
○	○	Retired Military ONLY - list current occupation below

→ You_____

→ Father_____

○	○	Civilian ONLY - list occupation below

→ You_____

→ Father _____

13. If MILITARY, what is your AND/OR the father's CURRENT rank/paygrade?

YOU

○ E-1	○ W-1	○ O-1
○ E-2	○ W-2	○ O-2
○ E-3	○ W-3	○ O-3
○ E-4	○ W-4	○ O-4
○ E-5	○ W-5	○ O-5
○ E-6		○ O-6
○ E-7	○ O-1E	○ O-7 or above
○ E-8	○ O-2E	
○ E-9	○ O-3E	○ Not applicable

FATHER

○ E-1	○ W-1	○ O-1
○ E-2	○ W-2	○ O-2
○ E-3	○ W-3	○ O-3
○ E-4	○ W-4	○ O-4
○ E-5	○ W-5	○ O-5
○ E-6		○ O-6
○ E-7	○ O-1E	○ O-7 or above
○ E-8	○ O-2E	
○ E-9	○ O-3E	○ Not applicable

SECTION B: GENERAL HEALTH

14. Do YOU have a history of any of the following:

	Yes	No	Don't know
a. Cancer	○	○	○
b. Heart disease	○	○	○
c. Diabetes	○	○	○
d. Gestational diabetes	○	○	○
e. Hypertension	○	○	○
f. Asthma	○	○	○
g. Thyroid problems	○	○	○
h. Gastrointestinal problems	○	○	○
i. Epilepsy	○	○	○
j. Other (specify_____)	○	○	○

15. Before this pregnancy, when was your last routine physical exam?

- ○ Within the previous year
- ○ 1-2 years before
- ○ 3-5 years before
- ○ Over 5 years before
- ○ Never

16. Have you ever smoked cigarettes?

- ○ No (continue at question 17)
- ○ Yes

→ If you no longer smoke, in which month and year did you quit? MO. ____ 19?? ____

→ How many cigarettes per day did/do you usually smoke? ____

17. Are you exposed to anyone's tobacco smoke for at least 30 minutes total per day?

	No	Yes
a. At home	○	○
b. At work	○	○
c. Elsewhere	○	○

18. Approximately how many servings (e.g., cups, glasses, cans) of caffeinated beverage (e.g., coffee, tea, soft drink) do you drink per day on average?

○ 0	○ 3	○ 6	○ 9
○ 1	○ 4	○ 7	○ 10
○ 2	○ 5	○ 8	○ More than 10

19. Are you currently taking any:

a. prescription drugs?
- ○ No
- ○ Yes, specify:_____

b. other medication (e.g., vitamins, over-the-counter pain relievers, sinus medication)?
- ○ No
- ○ Yes, specify:_____

20. Have you ever consumed alcohol?

- ○ No (continue at question 21)
- ○ Yes

→ One year prior to this pregnancy, in general how many drinks per week did you drink? ____ ○ None

→ If you currently drink, how many drinks per week do you usually drink? ____ ○ None

21. Please indicate which of the following you have ever used. (Mark ALL that apply.)
- ○ Marijuana
- ○ Cocaine
- ○ Heroin
- ○ Other, specify_____
- ○ None

SECTION C: DENTAL HISTORY

22. How many times per week do you brush your teeth?

- ○ 0
- ○ 1-6
- ○ 7 (meaning once a day)
- ○ 8-13
- ○ 14 (meaning twice a day)
- ○ More than 14

23. Do you routinely floss your teeth?

- ○ No (continue at question 24)
- ○ Yes

→ If Yes, please indicate how often:

- ○ Daily
- ○ Weekly
- ○ Monthly

24. Prior to pregnancy, did your gums bleed from brushing, flossing, and/or spontaneously?

- ○ No
- ○ Yes

25. Have you had any cavities within the last year?

- ○ No (continue at question 26)
- ○ Yes

→ If Yes, please indicate how many:

- ○ 1-3
- ○ 4-6
- ○ 7-10
- ○ More than 10

26. Have you lost any teeth within the last year (not due to injury or removal of wisdom teeth)?

- ○ No
- ○ Yes

27. Have you ever been told by a dentist that you have gum disease and/or any type of dental disease?

- ○ No
- ○ Yes

SECTION D: GYNECOLOGICAL/ OBSTETRICAL HISTORY

28. Prior to this pregnancy, were you using a method of birth control?

- ○ No (continue at question 29)
- ○ Yes

→ If Yes, please indicate which one(s). (Mark ALL that apply.)

- ○ Diaphragm
- ○ Condom
- ○ Foam/spermacides
- ○ IUD (Intrauterine Device)
- ○ Rhythm/any other natural family planning
- ○ Withdrawal
- ○ The Pill
- ○ Norplant®
- ○ Depo-Provera®
- ○ Hypodermic injections
- ○ Other (specify_____)

29. a. If YOU were on active duty at the time of conception, were you assigned to a ship?

○ No ○ Yes ○ N/A

b. If the FATHER of your baby was on active duty at the time of conception, was he assigned to a ship?

○ No ○ Yes ○ N/A

30. Have you ever had any of the following conditions?

	Yes	No	Don't know
a. Surgery involving fallopian tube(s)	○	○	○
b. Congenital (born with) tubal abnormality	○	○	○
c. Other congenital reproductive abnormality	○	○	○
d. Fertility treatment(s)	○	○	○
e. Chromosomal (genetic) abnormality	○	○	○
f. Positive (abnormal) pap smear	○	○	○
g. Endometriosis (abnormal growth of uterine lining)	○	○	○
h. Endometritis (uterine lining infection after pregnancy)	○	○	○
i. Abnormal estrogen/progesterone level	○	○	○
j. Blood-group incompatibility with fetus (Rh or other blood incompatibility)	○	○	○
k. Bleeding during pregnancy	○	○	○
l. "Bag of waters" breaking before the start of contractions	○	○	○
m. Other reproductive complication(s) OR problems, specify: _____	○	○	○
n. Chlamydia	○	○	○
o. Gonorrhea	○	○	○
p. Genital herpes	○	○	○
q. Syphilis	○	○	○
r. Trichomoniasis	○	○	○
s. Yeast infection	○	○	○
t. Vaginal infection	○	○	○
u. PID (Pelvic Inflammatory Disease)	○	○	○
v. Genital warts	○	○	○
w. Bacterial vaginosis	○	○	○
x. Chronic discharge and/or odor	○	○	○
y. HIV	○	○	○
z. Other, specify: _____	○	○	○

31. How many sexual partners have you had:

in the last 30 days?	in the last 6 months?
00	00
11	11
22	22
33	33
44	44
55	55
66	66
77	77
88	88
99	99

32. Within the last year (including current pregnancy), how frequently on average did you have intercourse?

- ○ Every day
- ○ 2-6 times a week
- ○ Once a week
- ○ Once a month
- ○ Less than once a month
- ○ Other

33. Have you ever douched in the last 12 months?

- ○ No (continue at question 34)
- ○ Yes

→ If Yes, when?

- ○ As regular hygiene
- ○ After sexual relations
- ○ As a result of vaginal itching or discharge (and/or fishy vaginal odor)

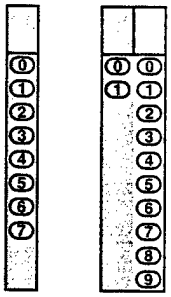
→ If Yes, what product(s) was used? (Mark ALL that apply.)

- ○ Water
- ○ Vinegar
- ○ Store bought
- ○ Other

SECTION E: PREGNANCY HISTORY AT ENROLLMENT

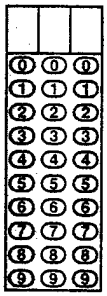
34. What is your current height?

Feet | Inches

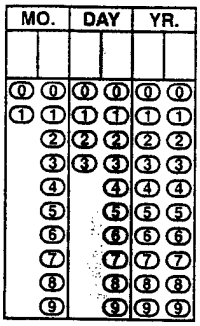


35. What was your pre-pregnancy weight?

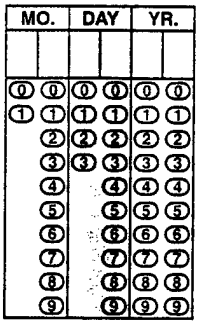
Pounds



36. What is your estimated delivery date?



37. What was the start date of your last menstrual period?



38. How many weeks pregnant are you?

Weeks

39. How many times have you been pregnant (including current pregnancy and previous abortions and miscarriages)?

- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7
- ○ 8
- ○ 9
- ○ 10
- ○ More than 10

If you answered "1" to question 39, STOP. You are finished with the questionnaire. Otherwise, continue at question 40.

40. If you have been pregnant before, what was the outcome each time you were pregnant? Each pregnancy (columns 1, 2, 3, etc.) should have only ONE outcome (item a, b, c, or d) marked per column.

Start here →

	Pregnancy									
	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth
a. live birth (children born alive)	○	○	○	○	○	○	○	○	○	○
b. still birth (fetal deaths occurring after 22nd week of pregnancy)	○	○	○	○	○	○	○	○	○	○
c. miscarriage/spontaneous abortion (fetal death before 22nd week of pregnancy)	○	○	○	○	○	○	○	○	○	○
d. abortion (pregnancy surgically terminated)	○	○	○	○	○	○	○	○	○	○

41. For each live birth pregnancy marked above in question 40a, follow the column down to indicate the outcome(s). (Mark ALL that apply to each pregnancy.)

a. full term birth	○	○	○	○	○	○	○	○	○	○
b. premature birth (born before 37th week of pregnancy)	○	○	○	○	○	○	○	○	○	○
c. low birth weight birth (less than 5 lbs 8 ozs)	○	○	○	○	○	○	○	○	○	○

42. If a pregnancy was terminated for medical reasons, please specify why (i.e., chromosomal defects, maternal complications, etc.):

_____

_____

_____

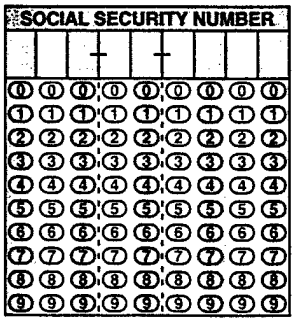
Thank you for your participation!

IMPORTANT INSTRUCTIONS

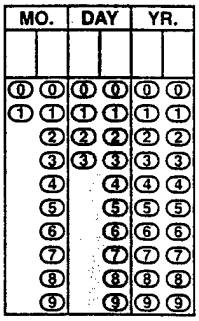
* USE NO. 2 PENCIL ONLY.
* Do NOT use ink, ballpoint, or felt tip pens.
* Erase cleanly and completely any changes you make.

SECTION G: First Prenatal Exam/Lab Results

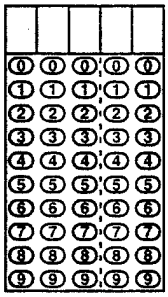
1. **SPONSOR'S Social Security Number?**



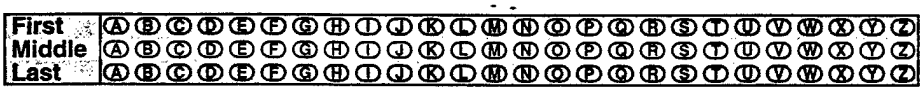
2. **SUBJECT'S birthdate?**



3. **STUDY ID #**



4. **SUBJECT'S initials?**



5. **Unsatisfactory specimen** ○ No ○ Yes **Date:** _____

Bacterial Vaginosis Slide: **Date:** _____

6. **Score (#):**

○	0	○	4	○	8
○	1	○	5	○	9
○	2	○	6	○	10
○	3	○	7		

		No	Yes
7.	Yeast	○	○
8.	Sperm	○	○

9. **WBC:**
○ absent
○ rare
○ few
○ moderate
○ many

Comments: _____

_____

_____

please continue on other side of form...

		Not Done	Done
10.	Wet Prep:	○	○

		No	Yes
11.	Trichomonas	○	○
12.	Clue Cells	○	○
13.	Yeast	○	○
14.	Sperm	○	○
15.	Red Blood Cells	○	○

16. WBC:
- ○ absent
- ○ rare
- ○ few
- ○ moderate
- ○ many

		Neg	Pos	Not Done
17.	Whiff test (KOH)	○	○	○
18.	InPouch® T.v.	○	○	○

Date: _____

		No	Yes
19.	Blood in Vagina	○	○
20.	Vaginal discharge present	○	○

Description:
- ○ clumpy
- ○ homogenous
- ○ frothy

21. pH:

○ Not Done

IMPORTANT INSTRUCTIONS

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* Erase cleanly and completely any changes you make.

SECTION H: Mother Data at Delivery

SPONSOR'S Social Security Number?

SOCIAL SECURITY NUMBER

SUBJECT'S birthdate?

MO.	DAY	YR.

STUDY ID #

SUBJECT'S initials?

First	Ⓐ Ⓑ Ⓒ Ⓓ Ⓔ Ⓕ Ⓖ Ⓗ Ⓘ Ⓙ Ⓚ Ⓛ Ⓜ Ⓝ Ⓞ Ⓟ Ⓠ Ⓡ Ⓢ Ⓣ Ⓤ Ⓥ Ⓦ Ⓧ Ⓨ Ⓩ
Middle	Ⓐ Ⓑ Ⓒ Ⓓ Ⓔ Ⓕ Ⓖ Ⓗ Ⓘ Ⓙ Ⓚ Ⓛ Ⓜ Ⓝ Ⓞ Ⓟ Ⓠ Ⓡ Ⓢ Ⓣ Ⓤ Ⓥ Ⓦ Ⓧ Ⓨ Ⓩ
Last	Ⓐ Ⓑ Ⓒ Ⓓ Ⓔ Ⓕ Ⓖ Ⓗ Ⓘ Ⓙ Ⓚ Ⓛ Ⓜ Ⓝ Ⓞ Ⓟ Ⓠ Ⓡ Ⓢ Ⓣ Ⓤ Ⓥ Ⓦ Ⓧ Ⓨ Ⓩ

Lab results obtained from medical record review

1. **VDRL:** ○ Non-reactive ○ Reactive ○ NA
2. **RH:** ○ Negative ○ Positive ○ NA
3. **Blood type:**
   - ○ A
   - ○ B
   - ○ AB
   - ○ O
4. **WBC count (mm³):**
5. **Differential count:**

Neut/ Segs % | **Bands %** | **Lymph %**

Eosin % | **Baso %** | **Monos %**

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Please indicate whether the following tests were positive or negative.

		Negative	Positive	NA
6.	**Gonococcus:**	○	○	○
7.	**Herpes:**	○	○	○
8.	**HIV:**	○	○	○
9.	**Chlamydia:**	○	○	○

If POSITIVE, indicate date screened:

MO.	DAY	YR.
0 0	0 0	0 0
1 1	1 1	1 1
2 2	2 2	2 2
3 3	3 3	3 3
4 4	4 4	4 4
5 5	5 5	5 5
6 6	6 6	6 6
7 7	7 7	7 7
8 8	8 8	8 8
9 9	9 9	9 9

Date of negative rescreen:

MO.	DAY	YR.
0 0	0 0	0 0
1 1	1 1	1 1
2 2	2 2	2 2
3 3	3 3	3 3
4 4	4 4	4 4
5 5	5 5	5 5
6 6	6 6	6 6
7 7	7 7	7 7
8 8	8 8	8 8
9 9	9 9	9 9

		Negative	Positive	NA
10.	**Group B Streptococcus:**	○	○	○

If POSITIVE, indicate date screened below.

MO.	DAY	YR.
0 0	0 0	0 0
1 1	1 1	1 1
2 2	2 2	2 2
3 3	3 3	3 3
4 4	4 4	4 4
5 5	5 5	5 5
6 6	6 6	6 6
7 7	7 7	7 7
8 8	8 8	8 8
9 9	9 9	9 9

11. Received treatment for Group B Streptococcus?

○ No
○ Yes
○ NA

12a. Gravida including this pregnancy:

0 0
1 1
2 2
3 3
4 4
5 5
6 6
7 7
8 8
9 9

12b. Parity:

0 0
1 1
2 2
3 3
4 4
5 5
6 6
7 7
8 8
9 9

13. Pregnancy history:

Live births	Still births	Miscarriages/ Abortions
0 0	0 0	0 0
1 1	1 1	1 1
2 2	2 2	2 2
3 3	3 3	3 3
4 4	4 4	4 4
5 5	5 5	5 5
6 6	6 6	6 6
7 7	7 7	7 7
8 8	8 8	8 8
9 9	9 9	9 9

→ **Of the live births, indicate how many were:**

Full term	Pre term
0 0	0 0
1 1	1 1
2 2	2 2
3 3	3 3
4 4	4 4
5 5	5 5
6 6	6 6
7 7	7 7
8 8	8 8
9 9	9 9

14. Fill in ONE of the following:

Mother's weight at delivery:

Pounds

0	0	0
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9

Mother's weight at last prenatal visit:

Pounds

0	0	0
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9

15. Route of delivery:

○ Vaginal
○ C-section

→ What were the indications for C-section?

○ Abnormal presentation
○ Cephalic/Pelvic Disproportion (CPD)
○ Fetal distress
○ Failed induction
○ Elective repeat
○ Other, specify: _____

16. Membranes intact?

○ Yes
○ No

→ If No, was there premature rupture of membranes?

○ Yes
○ No

→ If No, number of hours membranes ruptured (rounded to the nearest 30 minutes):

Hours: 00 01 02 03 04 05 06 07 08 09

Mins: 00 30

17. Was delivery:

○ Spontaneous labor delivery
○ Indicated delivery

→ If indicated:

	No	Yes
Labor?	○	○
Was labor induced?	○	○

Number of hours in labor: 00 01 02 03 04 05 06 07 08 09

18. Was there antepartum fever (99.6)?

○ No
○ Yes
○ NA

19. Was there amnionitis?

○ No
○ Yes
○ NA

→ If yes, please specify method of diagnosis:

_____

_____

20. Was there cervical dilation?

○ No
○ Yes
○ NA

→ If yes, specify number of cm: 00 01 02 03 04 05 06 07 08 09

21. Was there postpartum endometritis?

○ No
○ Yes
○ NA

22. Was the amniotic fluid meconium stained?

○ No
○ Yes
○ NA

23. Were there any antibiotics used during labor?

○ No
○ Yes
○ NA

→ If yes, specify: _____

_____

24. Were there any antibiotics used during pregnancy?

○ No
○ Yes
○ NA

→ If yes, specify: _____

_____

		No	Yes
25.	Was there abruption of the placenta?	○	○
26.	Was there placenta previa?	○	○
27.	Was there oligohydramnios?	○	○
28.	Was there polyhydramnios?	○	○
29.	Tocolytics (medication to reduce contractions)?	○	○

30. Please note any complications prior to labor:

_____

_____

_____

_____

31. Please note any complications during labor:

_____

_____

_____

_____

32. Please note any complications postpartum (include Postpartum Endometritis if available):

_____

_____

_____

_____

33. Was the infant diagnosed with intrauterine growth retardation?

○ No
○ Yes

34. Preexisting maternal IDDM (insulin-dependent diabetes mellitus)?

○ No
○ Yes

→ If yes, indicate number of years requiring insulin:

35. Gestational diabetes?

○ No
○ Yes

→ If yes, indicate: ○ diet controlled
○ insulin requiring

36. Chronic maternal hypertension?

○ No
○ Yes

→ If yes, indicate number of years:

37. Pregnancy-induced hypertension (preeclampsia)?

○ No
○ Yes

→ If yes, indicate: ○ mild
○ severe
○ NA

38. Was there eclampsia/HELLP syndrome?

○ No
○ Yes

39. Highest diastolic blood pressure during pregnancy:

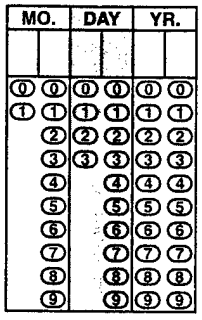
40. Highest urinary protein during pregnancy:

○ N
○ Trace (Tr)
○ +1
○ +2
○ +3
○ +4

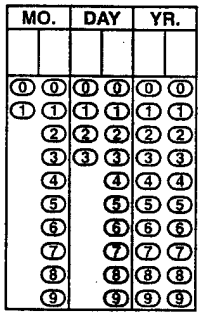
41. Was an ultrasound performed?

○ NA
○ No
○ Yes

→ If yes, date of first completed ultrasound:



→ If yes, estimated date of delivery given:



IMPORTANT INSTRUCTIONS

*** USE NO. 2 PENCIL ONLY.**
*** Do NOT use ink, ballpoint, or felt tip pens.**
*** Erase cleanly and completely any changes you make.**

SECTION I: Infant Data at Delivery

42. INFANT'S Social Security Number (Dependent Prefix and Sponsor SSN)

Prefix | SSN

43. MOTHER'S STUDY ID #

44. INFANT'S Date of birth

MO. | DAY | YR.

45. Military Time of birth

46. Number of children delivered:

- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5

47. If multiple birth, what is the infant number (e.g., twins = 1 or 2; triplets = 1, 2, or 3; etc.)?

- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5

48. Infant's sex:

- ○ Male
- ○ Female

49. Infant's race:

- ○ White/Caucasian
- ○ Black/African-American
- ○ Asian/Pacific Islander
- ○ Eskimo, Aleut, or American Indian
- ○ Spanish/Hispanic ancestry
- ○ Do not know
- ○ Other (specify: _____)

50. Infant admitted to:

- ○ Newborn Service
- ○ NICU
- ○ Both

51. Was infant:

	No	Yes	NA
SGA?	○	○	○
AGA?	○	○	○
LGA?	○	○	○

52. APGAR: 1 min: (0-10) 5 min: (0-10)

53. Infant's birthweight (grams):

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54. **What is the gestational age (in weeks) by obstetrical estimate?**

55. **What is the gestational age (in weeks) by Dubowitz?**

○ Not done

SECTION J: Infant Data at Discharge

56. **Date of discharge:**

MO.	DAY	YR.

57. **Infant death?**

○ No
○ Yes → Date of death:

MO.	DAY	YR.

→ If yes, cause of death:

_____

_____

58. **Was an autopsy performed?**

○ No
○ Yes
○ NA

→ If yes, were cultures taken?

○ No
○ Yes
○ NA

59. **Discharged to:**

○ Home
○ Other, specify: _____

60. **Did infant receive IVIG?**

○ No
○ Yes
○ NA

61. **Total days of hospitalization:**

Course of Oxygen Therapy

62. **Was oxygen given by bag-and-mask at delivery?**

○ No
○ Yes
○ NA

63. **Did any oxygen therapy take place after initial resuscitation?**

○ No
○ Yes

→ If yes, indicate number of days for each:

IPPV **NCPAP** **Oxyhood** **Cannula**

→ If yes, indicate total number of days oxygen required:

64. Type of oxygen at:

	IPPV	NCPAP	Oxyhood	Cannula
Less than 24 hrs	○	○	⊙	○
Day 1	○	○	○	○
Day 3	○	○	○	○
Day 5	○	○	○	○
Day 28	○	○	○	○

65. Was the infant on oxygen at 36 weeks GA?

○ No
○ Yes
○ NA

Clinical Parameters

Please indicate whether the following clinical parameters were present:

66. IVH (Intraventricular Hemmorrhage)

○ No
○ Yes
→ If yes, indicate grade: ○ Grade I ○ Grade II ○ Grade III ○ Grade IV

67. RDS (Respiratory Distress Syndrome)

○ No
○ Yes
→ If yes, did infant receive sufactant?

○ No
○ Yes
→ If yes, number of doses:

→ If yes, type:

_____

68. APNEA

○ No
○ Yes

69. BPD (Bronchopulmonary Dysplasia)

○ No
○ Yes

70. NEC (Necrotizing Entercolitis)

○ No
○ Yes
→ If yes, perforation?

○ No
○ Yes

71. PDA (Patient Ductus Arteriosus)

○ No
○ Yes

72. PVL (Periventricular Leukomalacia)

○ No
○ Yes

73. Transient tachypnea

○ No
○ Yes

74. Pneumonia

○ No
○ Yes

75. Airblock syndrome (i.e., pneumothorax, PIE)

○ No
○ Yes

76. Seizures

○ No
○ Yes

77. Meconium Aspirate Syndrome

○ No
○ Yes

78. Congenital anomalies

○ No
○ Yes
→ If yes, specify: _____

_____

79. Was sepsis suspected?

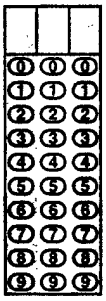
○ No
○ Yes
→ If yes, when (mark all that apply)?

○ 1st 48 hours
○ 1st 72 hours
○ 1st 7 days
○ After 7 days

80. Was the infant treated for suspected sepsis?

- ○ No
- ○ Yes
- ○ NA

→ If yes, duration (hours)?



81. Was hydrocephalus suspected?

- ○ No
- ○ Yes

→ If yes, when (mark all that apply)?

- ○ 1st 48 hours
- ○ 1st 72 hours
- ○ 1st 7 days
- ○ After 7 days

82. Was meningitis suspected?

- ○ No
- ○ Yes

→ If yes, when (mark all that apply)?

- ○ 1st 48 hours
- ○ 1st 72 hours
- ○ 1st 7 days
- ○ After 7 days

83. Was a CXR (chest x-ray) done?

- ○ No
- ○ Yes

→ If yes, were chronic lung changes noted on CXR?

- ○ No
- ○ Yes

→ If yes, give specifics and dates:

_____

_____

_____

_____

_____

_____

84. Was BPD (bronchopulmonary dysplasia)/ chronic lung disease found?

- ○ No
- ○ Yes

85. Were any antibiotics used on the infant?

- ○ No
- ○ Yes

→ If yes, please specify which:

_____

_____

_____

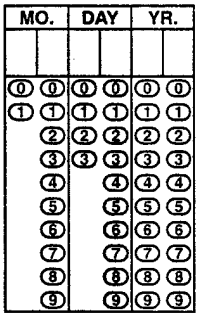
Test Procedures

86. Cord screen?

- ○ No
- ○ Yes
- ○ NA

→ If yes, indicate the following:

a. Date:



b. Blood type:

- ○ A
- ○ B
- ○ AB
- ○ O

	Negative	Positive
c. Rh:	○	○
d. Combs:	○	○
e. RPR (Syphylis):	○	○

Please CONTINUE with the rest of the "Test Procedures" questions. Use the separate form, "Part 2 Test Procedures".

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Part II Test Procedures

(Use as many additional forms as needed.)

SPONSOR'S Social Security Number

SSN

MOTHER'S STUDY ID #

MOTHER'S Date of birth

MO. DAY YR.

INFANT number (from question 47)

○ 1
○ 2
○ 3
○ 4
○ 5

87. WBC with differential Number of times test performed: _____

Date of test MO. DAY YR.	Total count (mm^3)	Lymph %	Monos %	Neut/ Segs %	Bands %	Baso %	Eosin %	% Other Immature granulocytes

Date of test (MO. / DAY / YR.)	Total count (mm^3)	Lymph %	Monos %	Neut/ Segs %	Bands %	Baso %	Eosin %	% Other Immature granulocytes

88. Urine for CMV (Cytomeglovirus)

Date of test

MO.	DAY	YR.

- Negative
- Positive

Date of test

MO.	DAY	YR.

- Negative
- Positive

Date of test

MO.	DAY	YR.

- Negative
- Positive

89. Blood cultures

Number of times test performed: ______

Date of test

MO.	DAY	YR.

Mycolplasmas
- Mycoplasma Species
- Uu

Bacteria ______________________

Virus ______________________

Antimicrobials ______________________

Date of test

MO.	DAY	YR.

Mycolplasmas
- Mycoplasma Species
- Uu

Bacteria ______________________

Virus ______________________

Antimicrobials ______________________

Date of test

MO.	DAY	YR.

Mycolplasmas
- Mycoplasma Species
- Uu

Bacteria ______________________

Virus ______________________

Antimicrobials ______________________

Date of test

MO.	DAY	YR.

Mycolplasmas
- Mycoplasma Species
- Uu

Bacteria ______________________

Virus ______________________

Antimicrobials ______________________

90. Endotracheal aspirates

Number of times test performed: ________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ________________

Chlamydia ________________

Antimicrobials ________________

CMV ________________

Respiratory viruses ________________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ________________

Chlamydia ________________

Antimicrobials ________________

CMV ________________

Respiratory viruses ________________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ________________

Chlamydia ________________

Antimicrobials ________________

CMV ________________

Respiratory viruses ________________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ________________

Chlamydia ________________

Antimicrobials ________________

CMV ________________

Respiratory viruses ________________

91. Cerebrospinal fluid

Number of times test performed: ________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Gram stain
- ○ Gram + cocci
- ○ Gram - cocci
- ○ Gram + rod
- ○ Gram - rod
- ○ Yeast
- ○ WBC
- ○ RBC
- ○ Negative

Bacteria ________________

Virus ________________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Gram stain
- ○ Gram + cocci
- ○ Gram - cocci
- ○ Gram + rod
- ○ Gram - rod
- ○ Yeast
- ○ WBC
- ○ RBC
- ○ Negative

Bacteria ________________

Virus ________________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Gram stain
- ○ Gram + cocci
- ○ Gram - cocci
- ○ Gram + rod
- ○ Gram - rod
- ○ Yeast
- ○ WBC
- ○ RBC
- ○ Negative

Bacteria ________________

Virus ________________

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Gram stain
- ○ Gram + cocci
- ○ Gram - cocci
- ○ Gram + rod
- ○ Gram - rod
- ○ Yeast
- ○ WBC
- ○ RBC
- ○ Negative

Bacteria ________________

Virus ________________

92. **Nasal pharangyl** **Number of times test performed:** ______

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ______

Chlamydia ______

Antimicrobials ______

CMV ______

Respiratory viruses ______

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ______

Chlamydia ______

Antimicrobials ______

CMV ______

Respiratory viruses ______

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ______

Chlamydia ______

Antimicrobials ______

CMV ______

Respiratory viruses ______

Date of test

MO.		DAY		YR.	
0	0	0	0	0	0
1	1	1	1	1	1
	2	2	2	2	2
	3	3	3	3	3
	4		4	4	4
	5		5	5	5
	6		6	6	6
	7		7	7	7
	8		8	8	8
	9		9	9	9

Mycolplasmas
- ○ Mycoplasma Species
- ○ Uu

Bacteria ______

Chlamydia ______

Antimicrobials ______

CMV ______

Respiratory viruses ______

PART II
Outcome Sheet for Non-Study Women

IMPORTANT INSTRUCTIONS

* **USE NO. 2 PENCIL ONLY.**
* **Do NOT use ink, ballpoint, or felt tip pens.**
* **Erase cleanly and completely any changes you make.**

SPONSOR'S
Social Security Number

SOCIAL SECURITY NUMBER

MOTHER'S
STUDY ID#

Date of Delivery

MO. DAY YR.

1. Outcome of study participant:

- ○ Known
- ○ Unknown → If unknown, why?
  - ○ Disenrolled
  - ○ Transferred-out
  - ○ Unknown disposition

2. Outcome of pregnancy:

a. Full-term birth (≥37 weeks gestation)
How many weeks?

b. Pre-term birth (<37 weeks gestation)
How many weeks?

c. Still birth/IUFD (fetal death after 22nd week of pregnancy)
How many weeks?

d. Miscarriage/SAB (fetal death before 22nd week of pregnancy
How many weeks?

e. Abortion/TAB (pregnancy surgically terminated)
How many weeks?

3. Route of delivery:

- ○ Vaginal
- ○ C-section abnormal presentation/breech
- ○ C-section failed inversion
- ○ C-section Cephalic-Pelvic Disproportion (CPD)/Macrosomia
- ○ C-section fetal distress
- ○ C-section failed induction
- ○ C-section elective repeat
- ○ C-section active phase arrest
- ○ C-section other (specify: _____)

continue on other side →

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4. Membranes intact?

○ Yes
○ No

→ If No, was there premature rupture of membranes?

○ Yes
○ No

→ If No, number of hours membranes ruptured (rounded to the nearest 30 minutes):

Hours	Mins
0 0	0 0
1 1	3 0
2 2	
3 3	
4 4	
5 5	
6 6	
7 7	
8 8	
9 9	

5. Was delivery:

○ Spontaneous labor delivery
○ Indicated delivery

→ If indicated:

	No	Yes
Labor?	○	○
Was labor induced?	○	○

Number of hours in labor:

0 0
1 1
2 2
3 3
4 4
5 5
6 6
7 7
8 8
9 9

6. Infant admitted to:

○ Newborn Service
○ NICU
○ Both
○ N/A

7. Maternal Complications:

GBS
○ Positive
○ Negative
○ Not tested

Chorio
○ Suspected
○ Not suspected
○ Not tested

Comments: _____

_____

_____

_____

_____

8. Neonatal Complications

Congenital Anomalies: _____

_____

_____

_____

IUGR: ○ Yes ○ No

APGAR:

1 min	5 min
0	0
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10

Birthweight:

Grams

0	0	0	0
1	1	1	1
2	2	2	2
3	3	3	3
4	4	4	4
5	5	5	5
6	6	6	6
7	7	7	7
8	8	8	8
9	9	9	9

Comments: _____

_____

_____

_____

_____

9. Bacterial Vaginosis Slide:

Date: _____

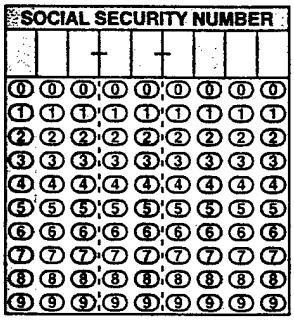
Score (#):
○ 0
○ 1
○ 2
○ 3
○ 4
○ 5
○ 6
○ 7
○ 8
○ 9
○ 10
○ Not Done

IMPORTANT INSTRUCTIONS

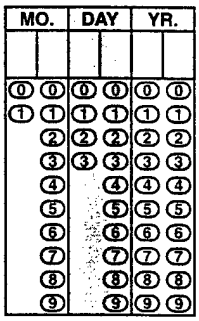
* **USE NO. 2 PENCIL ONLY.**
* **Do NOT use ink, ballpoint, or felt tip pens.**
* **Erase cleanly and completely any changes you make.**

Part II Lab Sheet

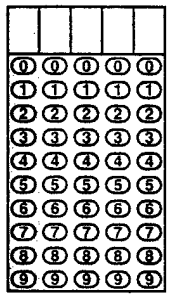
1. SPONSOR'S Social Security Number?



2. SUBJECT'S birthdate?



3. STUDY ID #



4. Vaginal swabs: ○ Done ○ Not done

5. BV smear: ○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10 ○ Not done

Placenta Culture Results:

6. Chorioamnion swab

- ○ Positive
- ○ Positive with contamination
- ○ Negative
- ○ Unsatisfactory

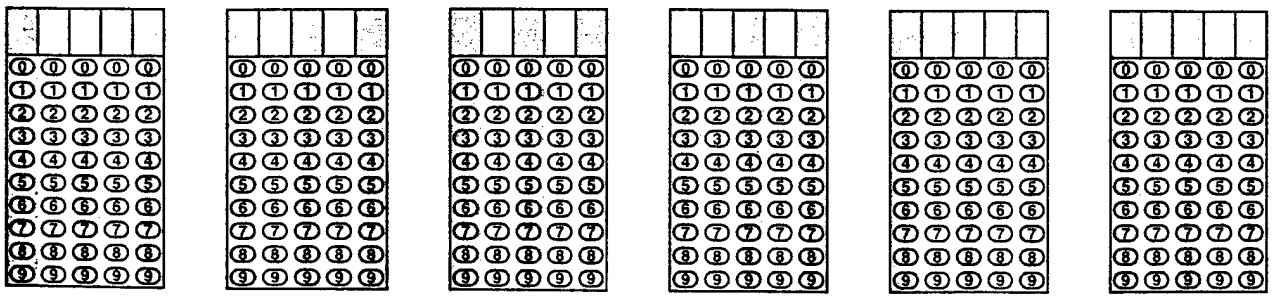
7. Placenta tissue

- ○ Positive
- ○ Positive with contamination
- ○ Negative
- ○ Unsatisfactory

8. Amniotic fluid

- ○ Positive
- ○ Positive with contamination
- ○ Negative
- ○ Unsatisfactory

9. List of bacteria:



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APPENDIX 3

Polymerase Chain Reaction Versus Culture: Detection Capability for *Ureaplasma urealyticum*

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*Department of Microbiology, University of Alabama at Birmingham, AL

ABSTRACT

Preterm birth (World Health Organization definition: birth less than 37 completed weeks gestation or low birthweight less than 2500 grams) is the single most common risk factor for infant morbidity and mortality, affecting 8-10% of all births and contributing to more than 60% of all perinatal morbidity and mortality. In a recent study, *Ureaplasma urealyticum* was found to be the single most common organism isolated from the chorioamnion of women in preterm labor with intact membranes. Ureaplasmal infection of the chorioamnion was significantly associated with chorioamnionitis, premature spontaneous labor and delivery, and development of postpartum endometritis.

Although risk factors for ureaplasmal colonization of the lower genitourinary tract have been identified, little information is available concerning risk factors for intrauterine infection and host immune responses to invasive infection. Development of disease in a subpopulation of infected individuals suggests that only certain subgroups of *U. urealyticum* may be pathogenic.

U. urealyticum has fourteen recognized serovars; these can be classified into two distinct genetic clusters known specifically as Biovar 1 and Biovar 2. Previously, we have developed monoclonal antibodies to a serovar-specific antigen (MB antigen), allowing differentiation of the fourteen serovars. Also, based on homology among sequences of the *mba*-gene from

several serovars, we have defined a set of polymerase chain reaction (PCR) primers (UM-3) that distinguish between the two biovars. The purpose of the present study was twofold: 1) to compare the efficacy of PCR using these primers to that of detection by culture of *U. urealyticum* in 43 clinical specimens and 2) to evaluate the use of these primers for biotyping 36 clinical isolates of *U. urealyticum*. Women who had delivered at the time of this study totaled 99; after excluding one set of twins, 97 deliveries (64 vaginal deliveries, 17 Cesarean with intact membranes, and 16 Cesarean with ruptured membranes) were evaluated by culture. Of 95 vaginal, 97 placental, 22 amniotic fluids, and 94 nasal swabs of infants, 59%, 21.6%,18%, and 23.4% were culture positive for ureaplasmas, respectively. 43 specimens from women who were positive for *U. urealyticum* in the upper tract were evaluated by PCR with the following results: 58.1% (25/36) concordance between PCR of the direct specimen and direct culture. In 36 isolates grown from positive culture, 91.6% (33/36) were positive by PCR for either Biovar 1 or Biovar 2. Overall, Biovar 1 was found more commonly than Biovar 2 in direct specimen PCR; Biovar 1 was found in 44.1% (19/43) of the specimens, Biovar 2 in only 13.9%(6/43). PCR of isolates found 83.3%(30/36) of specimens to have Biovar 1 and 33.3%(12/36) to have Biovar 2. Results suggest that culture and PCR methods are comparable in sensitivity for detection of *U. urealyticum*. Three of the samples were negative by PCR for both biovars, possibly indicating that a third biovar may exist that is undetectable with UM-3 primers. In conclusion, although PCR of known ureaplasma isolates is an effective method for verifying culture positives specimens, more capable primers may still need to be developed in order to implement this method of detection.

INTRODUCTION

The single most common risk factor for infant morbidity and mortality is preterm birth. Defined by the World Health Organization as less than 37 complete weeks gestation or birthweight less than 2500 grams, preterm birth affects 8-10% of all births and contributes to more than 60% of all perinatal morbidity and mortality. In the United States, spontaneous labor accounts for approximately 50% of preterm births with the remainder stemming from either preterm amnion rupture or delivery indicated for maternal medical or obstetric complications. If the pathophysiologal processes behind spontaneous preterm birth can be better understood, many of these young lives might be saved.

Ureaplasma urealyticum is a common commensal of the urogenital tract which occurs in up to 80% of sexually mature females (Figure 1). In a subpopulation of infected individuals, evidence indicates that ureaplasmas can also invade the upper genitourinary tract. Considered to be an important opportunistic pathogen during pregnancy, *U. urealyticum* was found in a recent study to be the single most common organism isolated from the chorioamnion of women in preterm labor with intact membranes delivered by Cesarean section. Ureaplasmal

infection of the chorioamnion was significantly associated with histologic chorioamnionitis, birth ≤ 34 weeks, and development of postpartum endometritis.

Although risk factors for ureaplasmal colonization of the lower genitourinary tract have been identified, little information is available concerning risk factors for intrauterine infection and host immune responses to invasive infection. Disease limited to a subpopulation of infected individuals suggests that only certain subgroups of *U. urealyticum* may be pathogenic. However, the development of reliable typing reagents is necessary to explore this possibility.

U. urealyticum has 14 recognized strains or serovars. These can be classified into two distinct clusters known specifically as Biovar 1 and Biovar 2. This mycoplasma can be identified by a multiple-banded (MB) surface antigen that also distinguishes the 14 serovar types (Figure 2). Cloning and sequencing of the mba-gene has revealed 3 distinct regions: an N-terminus signal peptide, a conserved region (biovar specific), and variable section containing nucleotide tandem repeats (serovar specific). This mba-gene provides a possible target for biotyping *U. urealyticum* through the use of monoclonal antibodies and polymerase chain reaction (PCR) primers. Thus, biovar typing of ureaplasmas by the mba-gene through the use of techniques such as PCR is an important step toward uncovering the organism's pathogenic potential.

PURPOSE OF THE STUDY

The purpose of the present study was twofold: 1) to compare the efficacy of PCR using UM-3 primers to that of detection by culture of *U. urealyticum* in 43 clinical specimens and 2) to evaluate the use of these primers for biotyping 36 clinical isolates of *U. urealyticum*.

METHODS AND MATERIALS

Study Population: Pregnant women (N=98) enrolled in an ongoing study of 12,000 women funded by Department of Defense, involving both the San Diego Naval Medical Hospital and The Department of Microbiology's Mycoplasma Laboratory at The University of Alabama at Birmingham, were participants in this study. At the first prenatal visit, vaginal swabs were performed to screen for the presence of *U. urealyticum*.

At the time of delivery, specimens were obtained of the mother's vagina, chorioamnion, and amniotic fluid. Additionally, a nasal swab from the infant was also obtained.

Specimens were frozen immediately in San Diego in dry ice at approximately -70°C and shipped express mail to Birmingham, Alabama for analysis.

Isolation of Ureaplasmas: Media (10B, A8, and SP4 broth and agar) were prepared and underwent quality control. All specimens previously mentioned were thoroughly mixed on a vortex mixer in 10B. Four tenfold dilutions were made from a 100 -µl aliquot of each specimen type in 10B and SP4 broths to minimize mycoplasmacidal tissue factors known to interfere with culture recovery of

mycoplasmas (vaginal and nasal specimen were diluted three times in 10B only). An aliquot (20µl) of the undiluted specimen and each dilution were inoculated onto A8 and SP4 agar. All plated media were incubated at 37°C under 5% carbon dioxide and 95% nitrogen in a humidified incubator for a minimum of 30 days before they were designated negative. All broths were incubated at 37°C under atmospheric conditions. Any broth tube showing color change suggestive of mycoplasmal growth was subcultured to solid media and incubated further. Colonies of *U. urealyticum* were identified on A8 agar by urease production in the presence of calcium chloride indicator. These brown or black colonies were then inoculated into 10 ml of 10B. Once the ureaplasmas were growing at log phase, (about 16 hours or 10B media changing color), the culture was stepped up and grown in 15ml of 10B, subsequently divided into 1 ml aliquots, and stored at -80°C.

PCR Reactions: The ureaplasma from each 1 ml stock culture of 43 cultured specimens was harvested by centrifugation. The pelleted ureaplasmas were then resuspended in 100 µl or proteinase K buffer (0.25 mg of proteinase/ml) and incubated for 1 hour at 60°C prior to heat inactivation at 95°C for 10 minutes. This sample was then used for PCR.

The amplification reaction mixtures contained 50 µl of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin, 1.25 U of Taq polymerase (Perkin-Elmer, Norwalk, Conn.), 200 µM (each) deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP; Perkin-Elmer), 0.8 µM (each) primer and 5 µl of sample.

With the UM-3 PCR primers (Figures 4 and 5), both the external and internal reactions were carried out in a DNA thermal cycler (Thermolyne, Dubuque, Iowa) with a beginning denaturation step (94°C, 2 min); 40 cycles of denaturation (94°C, 20 sec), annealing (58°C, 1 min), and extension (72°C, 2 min); and final extension step (72°C, 10 min).

RESULTS AND DISCUSSION

Cultural Isolation of Ureaplasmas: Of the 97 pregnant women who had delivered at the time of this study (64 vaginal, 16 Cesarean with ruptured membranes, and 17 Cesarean with intact membranes), 61 presented with positive cultures of *U. urealyticum* in at least one specimen. Positive culture results with respect to specimen type are summarized in Figure 3. 59% of vaginal, 21.6% of placental, 18% of amniotic fluids, and 23.4% of infants were culture positive for ureaplasmas. Twenty-one females with ureaplasmas present in the lower genital tract were positive in the upper tract as well. Colonization of ureaplasmas throughout pregnancy is indicated by the comparable percentages of prenatal vaginal and delivery vaginal cultures (independent of delivery type). Specifically, 89.3% (50/56) of vaginal deliveries had a persistence of *U. urealyticum* in the vagina throughout pregnancy (persistence in Cesarean deliveries were even higher).

Figure 3 also displays *U. ureaplasma* frequency in the chorioamnion, amniotic fluid, and infant. Cesarean deliveries with intact membranes are in striking contrast to those that delivered with ruptured membranes (as well as vaginal deliveries). This may be attributed to the incidental decreased colonization of ureaplasmas in women enrolled in the study that delivered with intact

membranes. It also supports evidence of the controlled environment intact membrane deliveries offer in studying the invasion characteristics of *U. urealyticum*.

PCR Versus Culture: Digitized images of PCR raw data are shown in Figures 4 and 5. Results of PCR are shown in Table 1 (vaginal deliveries) and Table 2 (Cesarean with ruptured membranes). Comparison of PCR and culture (see Figure 6) revealed that in detection of *U. urealyticum* in direct specimens, culture was a more reliable method (PCR agreed with culture 58.1% of the time). Vaginal and infant nasal samples, in particular, indicated that culture had a 50% greater efficacy in detecting ureaplasmas; chorioamnion specimens, however, were quite comparable.

Biotyping by PCR (Figures 7 and 8): Eight patients that had positive culture results for *U. urealyticum* in the upper tract were selected for biotyping by PCR. Overall, Biovar 1 was found more commonly than Biovar 2. Results of direct specimen PCR indicate that Biovar 1 was found in 44.1% (19/43) specimens, compared to Biovar 2 in only 13.9% (6/43). PCR of isolates revealed 83.% (30/36) had Biovar 1, yet only 33.3% (12/36) contained Biovar 2. These results suggest that PCR of isolates provides adequate detection of *U. urealyticum*; efficacy of these primers for use in direct specimens, however, may still need refinement.

Overall, PCR is comparable to that of detection by culture (91.6% detection of either Biovar 1 or Biovar 2 (30/36)).

CONCLUSIONS

1) The persistence of *U. urealyticum* during pregnancy in this study sample is high, irrespective of delivery type (Cesarean section with intact membranes, Cesarean section with ruptured membranes, or vaginal delivery). This is important because of the asymptomatic nature of *U. urealyticum* and its detrimental effects in low birthweight pregnancies.
2) Of the two methods of detection for *U. urealyticum* in the original specimen, our results indicate that culturing is a more sensitive than PCR. PCR agreed with culture in only 58.1% of the direct specimens.
3) Biovar 1 had a higher frequency of detection than Biovar 2. In 43 specimens evaluated by PCR, Biovar 1 was found in 44.1%(19/43) of the direct specimens as compared to Biovar 2 in 13.9%(6/43). In 36 isolates, 83.3%(30/36) contained Biovar 1 and 33.3%(12/36) had Biovar 2. This may be of clinical relevance because one of the biovars has been suspected to be more pathogenic.
4) The efficacy of biotyping using PCR is approximately 91.7% in detecting Biovar or Biovar 2. However, 8.3% of PCR results failed to indicate the presence of either biovar in specimens culturally positive for ureaplasmas. This evidence suggests the possibility that a separate biovar may exist that is not detectable with present PCR biotyping primers.

PCR RESULTS
(VAGINAL DELIVERIES)

Vaginal Deliveries						
Biovar 1	Specimen type		**patient #5 (10042)**	**patient #6 (10077)**	**patient #7 (10096)**	**patient #8 (10278)**
Prenatal Visit:	**Vagina**	Direct Specimen	neg	neg	pos	pos
		isolate	neg	pos	pos	pos
Delivery:	**Vagina**	Direct Specimen	neg	pos	neg	neg
		isolate	neg	pos	pos	pos
	Membrane	Direct Specimen	neg	neg	neg	pos
		isolate	neg	not grown	pos	not grown
	Tissue	Direct Specimen	neg	neg	pos	pos
		isolate	not grown	pos	pos	pos
	Amniotic Fluid	Direct Specimen	no specimen	no specimen	no specimen	no specimen
		isolate	no specimen	no specimen	no specimen	no specimen
	Nasal	Direct Specimen	neg	neg	pos	pos
		isolate	neg	pos	pos	pos
Biovar 2						
Prenatal Visit:	**Vagina**	Direct Specimen	neg	neg	neg	neg
		isolate	pos	neg	neg	neg
Delivery:	**Vagina**	Direct Specimen	neg	neg	neg	pos
		isolate	pos	neg	neg	pos
	Membrane	Direct Specimen	neg	neg	neg	pos
		isolate	neg	not grown	neg	not grown
	Tissue	Direct Specimen	neg	neg	neg	neg
		isolate	not grown	neg	neg	pos
	Amniotic Fluid	Direct Specimen	no specimen	no specimen	no specimen	no specimen
		isolate	no specimen	no specimen	no specimen	no specimen
	Nasal	Direct Specimen	neg	neg	neg	pos
		isolate	neg	neg	neg	pos

Table 1: Raw data of PCR results for vaginal deliveries (see corresponding Figures 4 and 5)

PCR RESULTS
(CESAREAN RUPTURED DELIVERIES)

C-section Deliveries (ruptured)						
Biovar 1	Specimen type		**patient #1 (10217)**	**patient #2 (10298)**	**patient #3 (10191)**	**patient #4 (10005)**
Prenatal Visit:	**Vagina**	Direct Specimen	pos	pos	neg	neg
		isolate	pos	pos	neg	pos
Delivery:	**Vagina**	Direct Specimen	neg	pos	neg	neg
		isolate	pos	pos	neg	pos
	Membrane	Direct Specimen	pos	neg	neg	neg
		isolate	pos	not grown	not grown	pos
	Tissue	Direct Specimen	neg	pos	pos	pos
		isolate	pos	not grown	not grown	pos
	Amniotic Fluid	Direct Specimen	no specimen	pos	neg	pos
		isolate	no specimen	pos	pos	pos
	Nasal	Direct Specimen	pos	pos	neg	neg
		isolate	pos	pos	pos	pos
Biovar 2						
Prenatal Visit:	**Vagina**	Direct Specimen	pos	pos	neg	neg
		isolate	pos	neg	neg	pos
Delivery:	**Vagina**	Direct Specimen	neg	neg	neg	neg
		isolate	neg	neg	pos	neg
	Membrane	Direct Specimen	neg	neg	neg	neg
		isolate	neg	not grown	not grown	pos
	Tissue	Direct Specimen	neg	neg	neg	neg
		isolate	neg	not grown	not grown	neg
	Amniotic Fluid	Direct Specimen	no specimen	pos	neg	neg
		isolate	no specimen	pos	pos	neg
	Nasal	Direct Specimen	neg	neg	neg	neg
		isolate	neg	neg	pos	neg

Table 2: Raw data of PCR results for Cesarean ruptured deliveries (see corresponding Figures 4 and 5)

Figure 1: Photograph of *Ureaplasma Urealyticum* (arrow) and *Mycoplasma Hominis*. Mycoplasmas are the smallest free living organism and unlike many other unicellular organisms, they do not have a cell wall.

MBA GENE

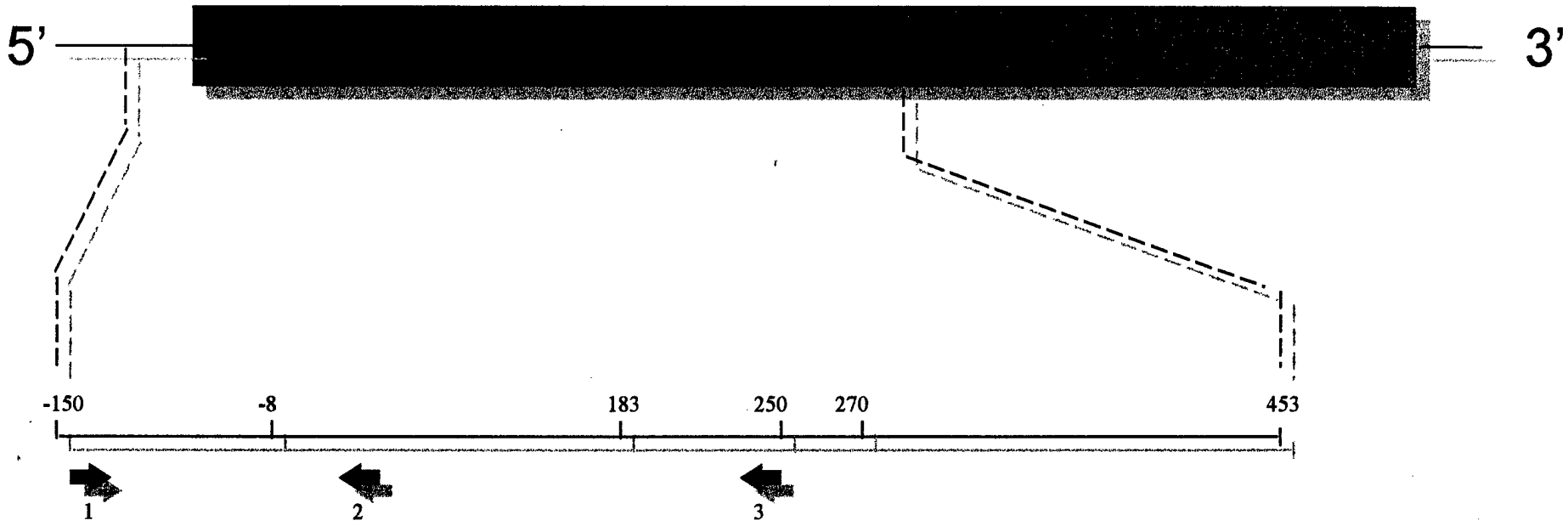


Figure 2: Schematic Map of the *U. Urealyticum* mba-gene and position of UM-3 PCR primers. Arrows indicate the approximate positions and orientations of the PCR primers. The gene consists of a region encoding a signal peptide(green), a conserved region, and a variable region. Primer1: UBS-1 (for Biovar 1) ar UBS-2 (for Biovar 2). Primer 2: UU-64bio1 (for Biovar 1) or UU64bio2 (for Biovar 2). Primer 3: UMA-226.

CULTURE RESULTS BY DELIVERY TYPE

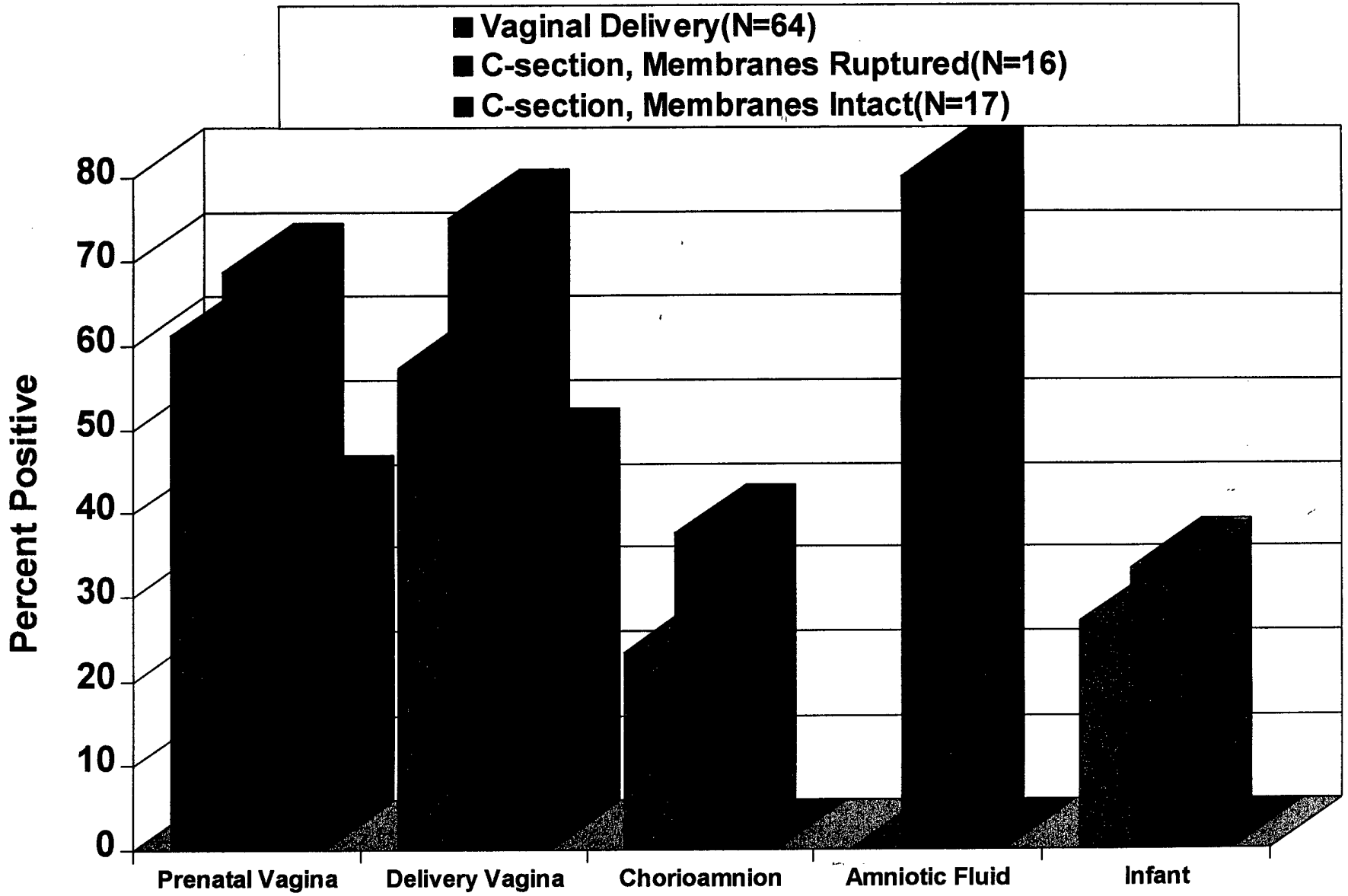
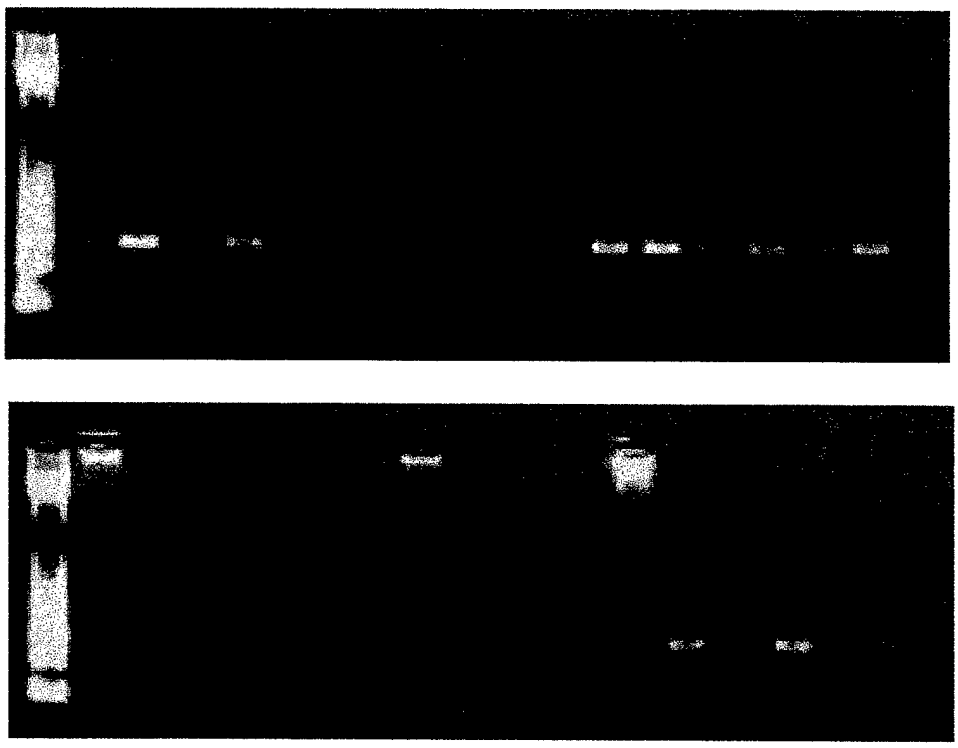


FIGURE 3:Culture positive results expressed as percent positive

FIGURE 4

Biovar I



Biovar II

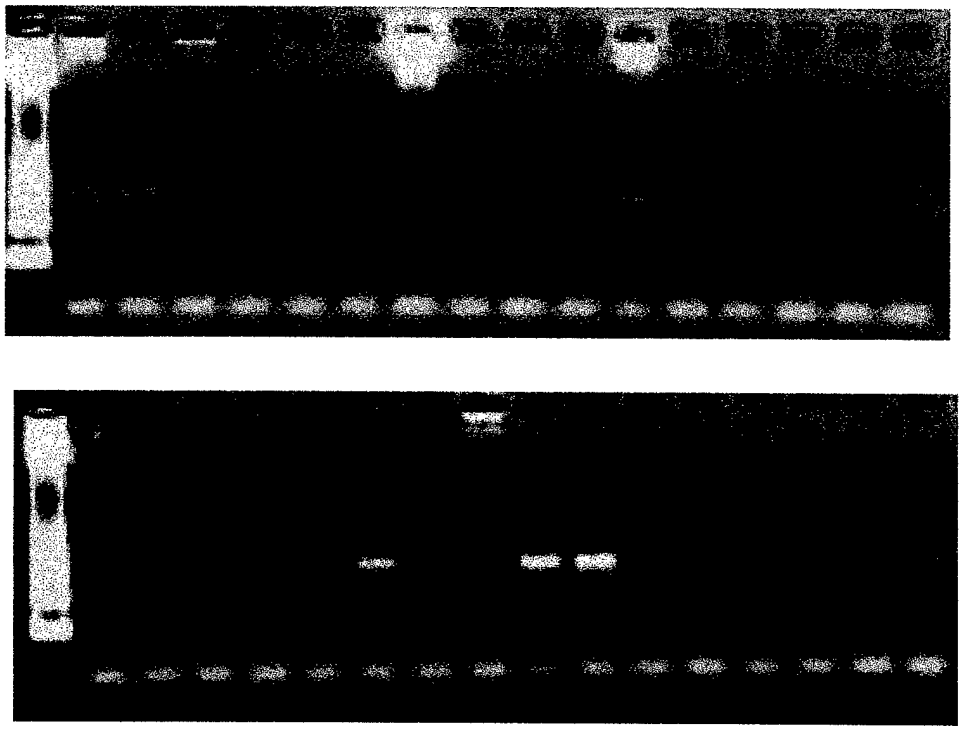
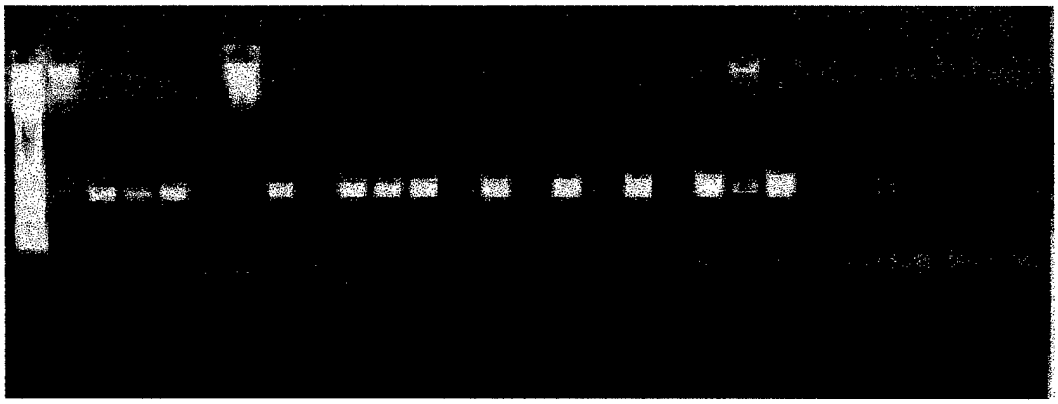
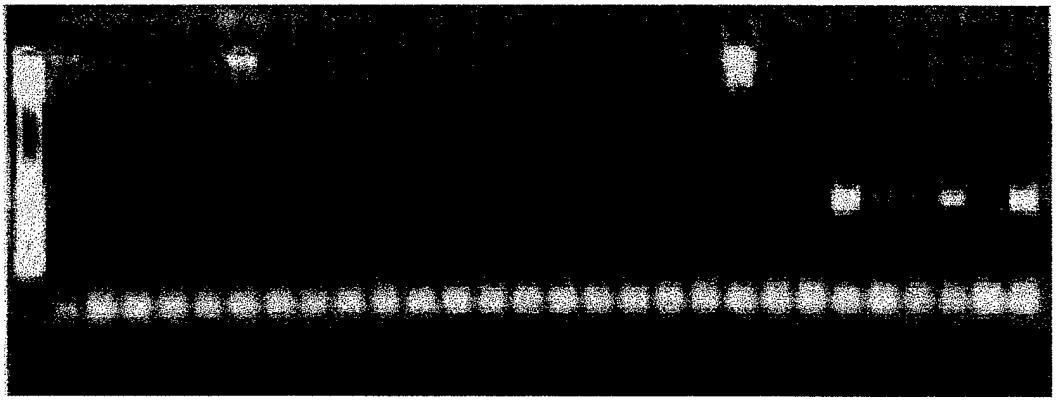


FIGURE 5

Biovar I



Biovar II



PCR VERSUS CULTURE

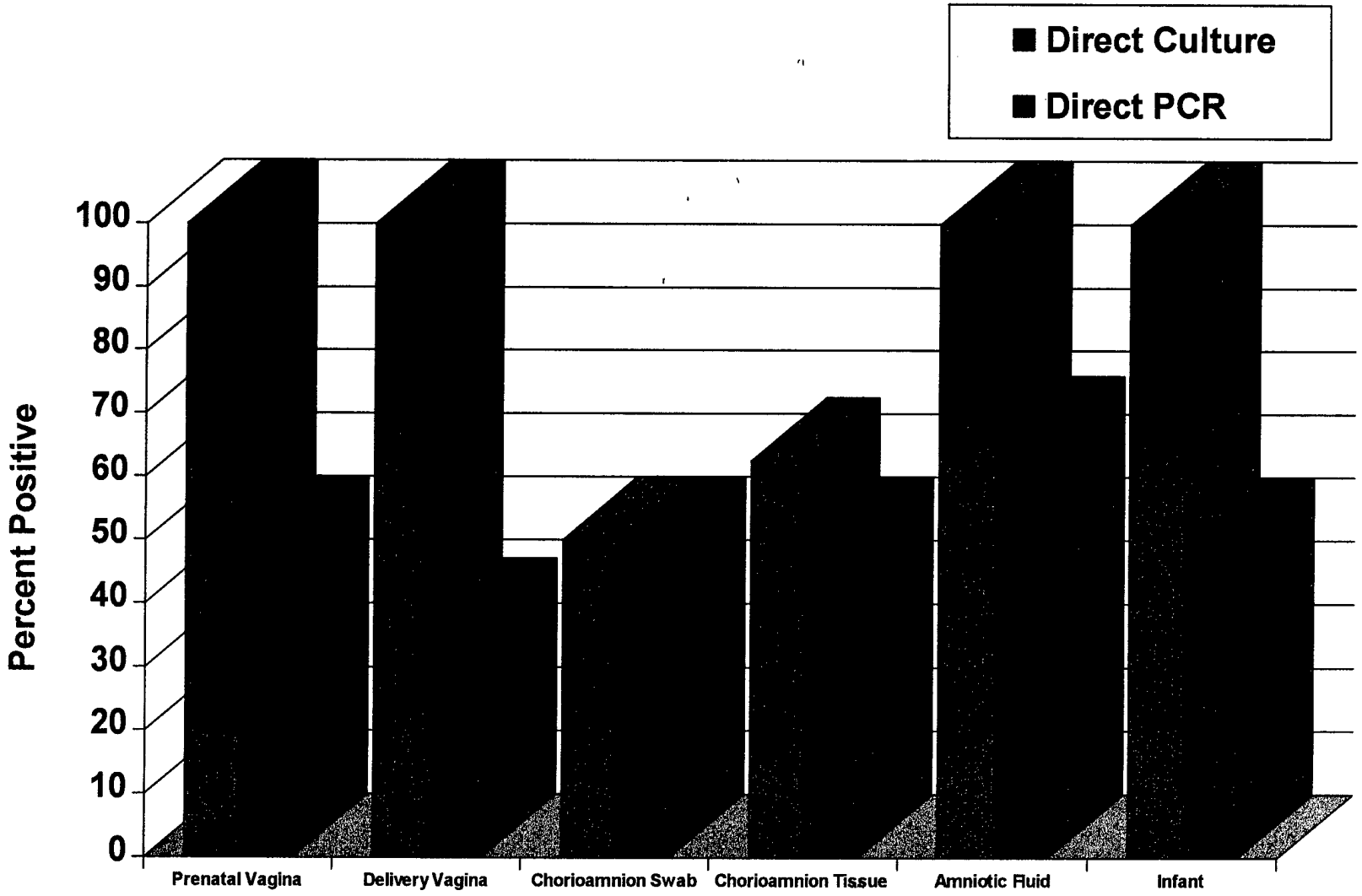


Figure 6: Comparison of PCR versus culture for detection of *U. urealyticum* in clinical specimens

PCR RESULTS OF BIOVAR I

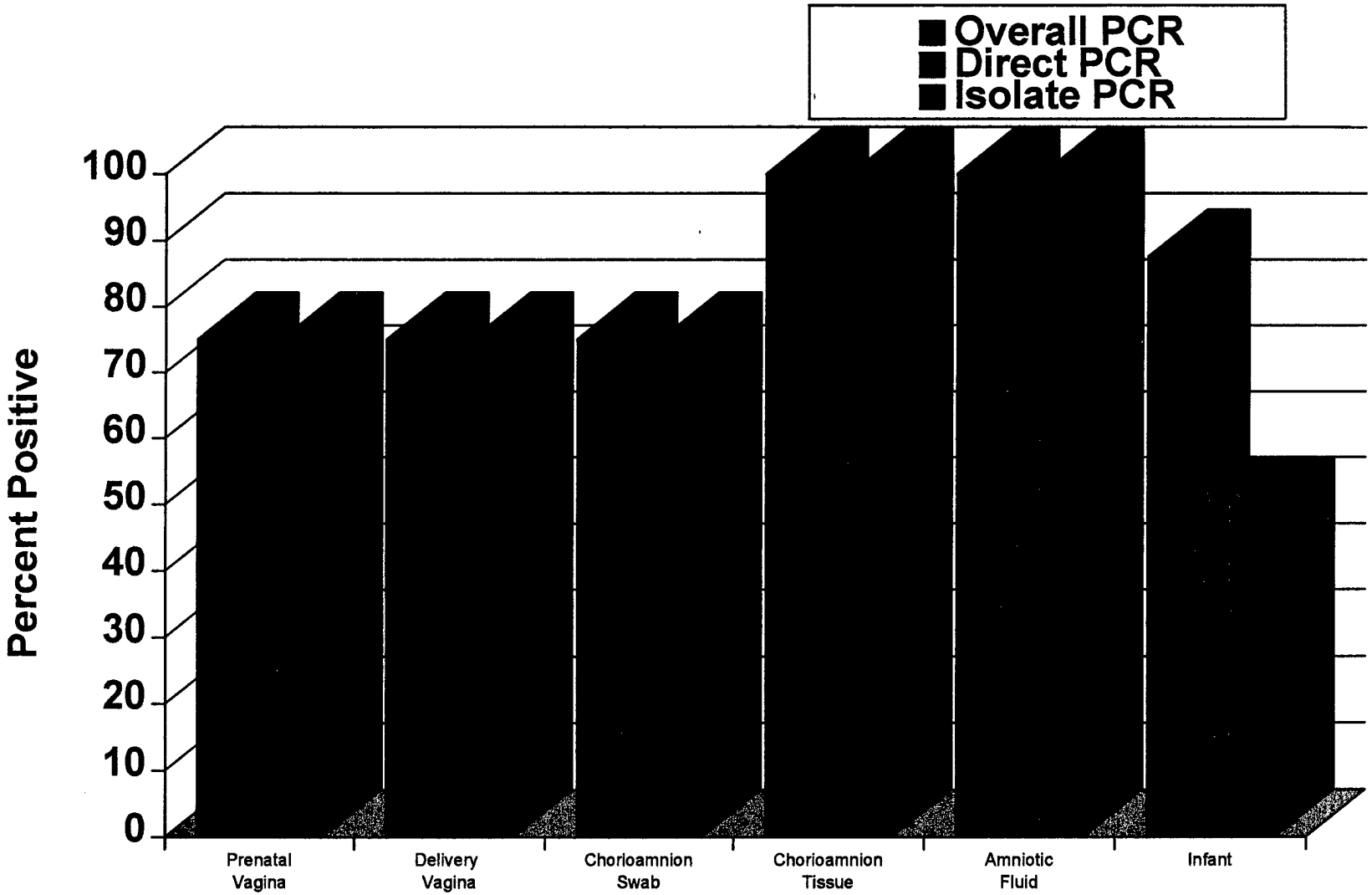


FIGURE 7: Biovar 1 positive direct and isolate specimens with overall Biovar 1 also shown (red)

PCR RESULTS OF BIOVAR II

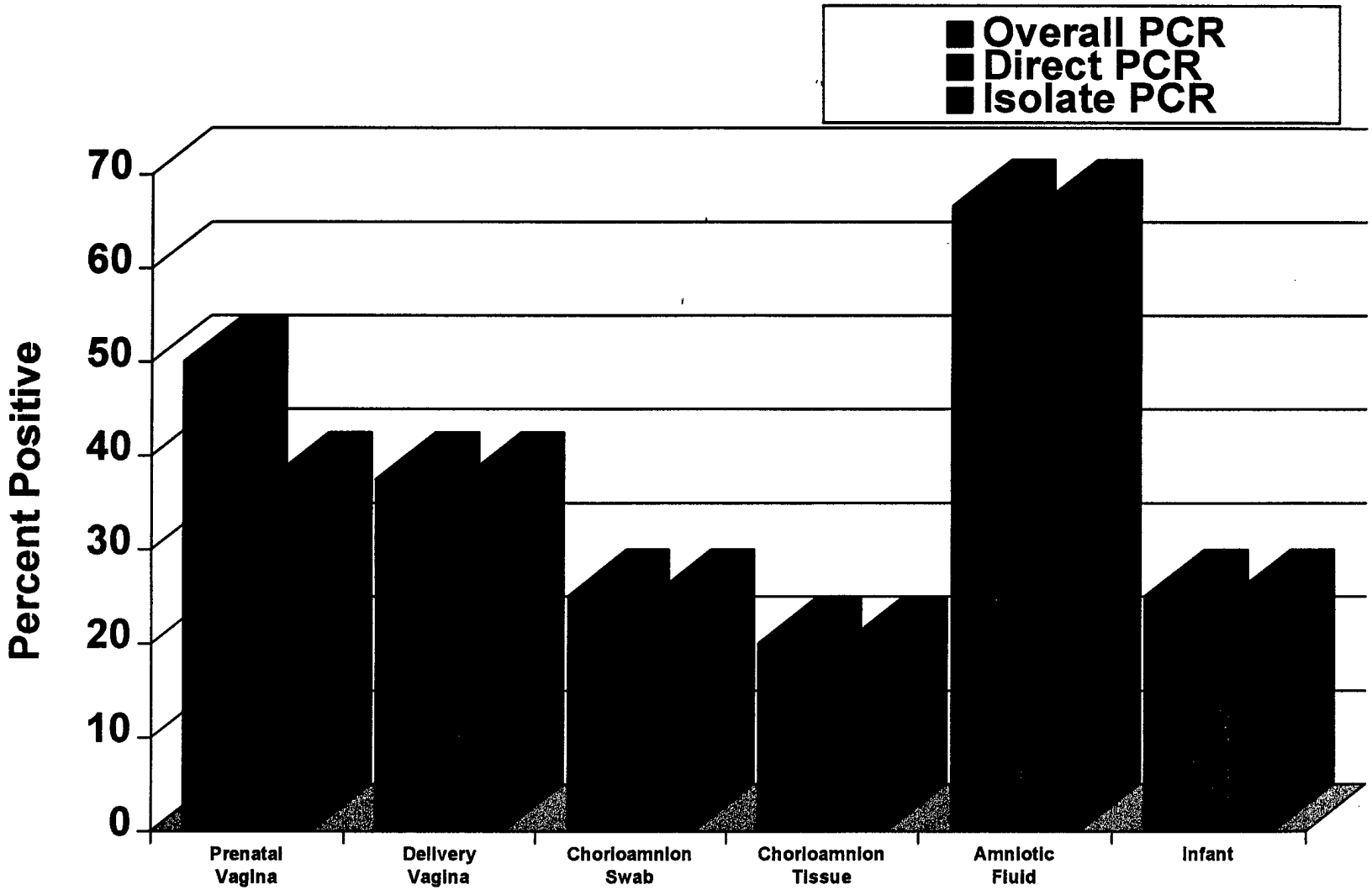


FIGURE 8: Biovar 2 positive direct and isolate specimens with overall Biovar 2 also shown (red)

APPENDIX 4

Date: 06/19/97

To: 3 WEST STAFF

From: UAB STUDY STAFF

Subject: UAB Chorioamnion Infection Study Update

The UAB study is under way and you may be wondering what is it all about ? Hopefully, we can answer some of your questions.

The objectives of the UAB Chorioamnion Infection Study are to determine whether *Ureaplasma urealyticum* Chorioamnion colonization is associated with preterm birth. We are also hoping to identify factors that may predict Chorioamnion invasion and preterm birth.

The UAB study began enrolling patients last June. We have currently enrolled over 1,260 women. Of these women, approximately 60% are colonized by Ureaplasma urealyticum and about 20% of our population has Bacterial Vaginosis. What does all this mean????

Occasionally, you may see a UAB patient prior to delivery for instance women in preterm labor or with premature ruptured membranes. It is very important when these women return to the Labor deck that L&D is aware of their UAB study status. It would be a great help to the study if this information gets passed along during report. The patients chart should have a blue UAB sticker on the front cover (although this isn't always the case). Extra blue UAB stickers may be found in the UAB binder at the 3 west front desk. Also included in the UAB binder are the research proposal (condensed version) and an extensive literature review.

Thanks for your interest in the UAB study! Hopefully, all the effort will be rewarded with improved maternal and neonatal outcomes. Please call us if you have any questions at 2-9242 or 979-3609.

Mara Berzins RN, MPH

Paul Stamper, Clinical Microbiologist

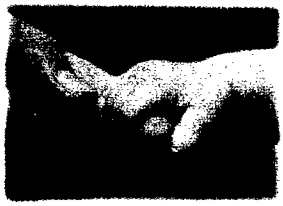
UAB STUDY BABIES:

IF YOU OBTAIN BLOOD CULTURES OR CSF DON'T FORGET A SAMPLE FOR UAB STUDY

MEDIA TUBES FOR BLOOD AND CSF ARE LOCATED IN THE MEDICATION REFRIGERATOR

WARM MEDIA TO ROOM TEMPERATURE BEFORE ADDING 0.2-0.5 ML SPECIMEN

CALL 29242 OR 979-2087 FOR QUESTIONS OR MEDIA PICK-UP



UAB STUDY

The Naval Medical Center, San Diego wishes to provide the best obstetrical care possible to military families. For this reason Naval Medical Center is collaborating with the **University of Alabama at Birmingham** and the Naval Health Research Center to investigate reports that infection causes premature labor. The results of this study may aid in the prevention of poor outcomes of pregnancy (premature birth, sickness and death of infants) among military active-duty and dependent women.

You have the opportunity to participate in this very important research study being conducted here at the Naval Medical Center, San Diego. The purpose of this study is to determine if a bacteria found in some women's vaginas or placentas is associated with premature delivery and infections in newborn infants.

If you choose to participate you will be asked:

At today's visit:

- to read and sign a consent form
- to fill out a data questionnaire
- to give about 2 teaspoons of blood while having your routine prenatal labs drawn
- to self-swab your vagina with small cotton swabs (similar to placing tampon)

Upon admission to labor and delivery:

- to give about 2 teaspoons of blood while having your routine admission labs drawn
- to have vaginal swabs collected by a L&D nurse or provider during 1st vaginal exam

At or after delivery you **MAY HAVE** specimens collected for further culture work including:

- about 2 teaspoons of amniotic fluid (this **DOES NOT** involve an amniocentesis)
- placental tissue
- umbilical cord blood
- nasal swab on infant

IF your infant should become ill and the Pediatrician orders any special tests on your infant, samples may be sent to UAB lab for culture.

There is **NO** increased risk for you or your baby by participating in this study. Your participation in this study **WILL NOT** interfere with or delay the normal care you and your baby receive. And, these procedures **WILL NOT** increase you or your baby's discomfort. This study **may** help you and other women have **healthier pregnancies** and **healthier babies**, in the future.

ALL information about you and your baby **WILL BE** kept confidential. Study data and specimens will be marked only with the last four digits of your sponsor's social security number and an assigned study number.

You will be asked to participate in this important study by study staff. **IF YOU HAVE ANY QUESTIONS PLEASE ASK**. By agreeing to participate in this study, you will make a tremendous contribution to the future understanding of how bacterial infections affect pregnancy outcome and the health of the newborn infant.

08 October 1996

Joel B. Lench, M.D., F.A.C.O.G.
Medical Director, Nurse Midwifery Service
San Diego Naval Medical Center
San Diego, CA 92134
(619)591-3107

CDR Gregory C. Gray MC
Naval Health Research Center
P.O. Box 85122
San Diego, CA 92186-5122
(619)553-9967

CDR Gray,

I am writing in regards to the possibility of pregnant women "self-swabbing" at their preregistration appointment when enrolled into the study "Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome Among Active Duty Military Women and Dependent Women." I do not feel any risk is imposed by pregnant women "self-swabbing" for vaginal cultures as long as proper instruction is provided.

Sincerely,

Joel B. Lench, M.D.

UAB STUDY
SELF-SWABBING INSTRUCTIONS

Self swabbing can be performed by standing with one foot propped on the toilet seat or by sitting on toilet with knees apart.

You have been given 6 sterile swabs (2 per package) and 1 sterile blue top glass tube.

1. Wash hands well with soap and water. Rinse well.
2. Cleanse outer vaginal area as instructed for "clean catch" urine specimen.
3. Very carefully remove 2 swabs from their packet (without touching the swab tip).
4. With one hand, gently hold open the skin surrounding the vaginal opening.
5. Swab the side walls of the vagina, be sure that swabs are **moist** with secretions.
6. Carefully place swabs in the sterile glass tube (**tips down**) without touching them to any other surface.
7. Repeat steps 3 through 7 for remaining swabs and replace blue top on sterile glass tube.
8. Obtain urine specimen as directed by Lab.
9. Return tube with all 6 swabs and urine specimens to the Lab.

DATE: 24 March 1997

FROM: UAB Study Staff

TO: OB/GYN Prenatal Healthcare Providers

SUBJECT: UAB Study Participant Self-Swabbing

As of Monday, March 24, 1997 study participants will be self-swabbing. This means providers will no longer be required to obtain vaginal swabs on study participants, who have enrolled as of March 24, 1997.

There are study participants who have enrolled *prior* to March 24, 1997 or who have had prior missed swabs and will require provider obtained vaginal swabs. If a patient needs swabs obtained there will be a UAB lab slip and/or missed swab reminder on the chart. By the end of April it will be rare for you to see a UAB study participant on their first prenatal visit who requires vaginal swabs.

We realize obtaining the swabs has been an added inconvenience to your already busy schedule and we appreciate the great effort. Thanks again!

DATE: 07 APRIL 1997

FROM: UAB Chorioamnion Infection Study Staff

TO: OB/GYN Prenatal Healthcare Providers

SUBJECT: UAB Study Participant Self-Swabbing Update

Study Participants have been self-swabbing as of March 24, 1997. This means, as a provider, you no longer have had to obtain first prenatal visit vaginal swabs on study participants, who have enrolled as of March 24.

There are study participants who have enrolled *prior* to March 24, 1997 or who have had prior missed swabs and still need vaginal swabs. If a patient needs swabs obtained there will be a UAB lab slip and/or missed swab reminder on the prenatal chart. You may obtain these vaginal swabs or direct the participant to the UAB study nurse for self-swabbing.

By the end of April, it will be unusual for you to encounter a UAB Study participant on their first prenatal visit who requires vaginal swabs.

Thanks in advance for collecting the last of the provider obtained prenatal vaginal swabs. Please call if you have any questions or concerns. We may be reached at 2-9242 or 979-3609.

UAB STUDY UPDATE

The UAB Study is well under way. We have already enrolled more than 1,650 women over the last 16 months with 375 deliveries. Since self-swabbing began in March, our enrollment has more than doubled. Beginning in October, approximately 50% of the women delivering here will be UAB study participants. For those of you who are not familiar with the study, this update may help answer some questions that you may have about the study.

The University of Alabama at Birmingham (UAB) in collaboration with the Naval Health Research Center (NHRC) and the Naval Medical Center, San Diego (NMCSD), is conducting a study, "**Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcomes Among Active-Duty Military and Dependent Women.**" Dr. Gail Cassell, Professor and Chairman of the Department of Microbiology at UAB was awarded a grant from the Department of Defense to study this issue. Dr. Cassell has been studying *Ureaplasma urealyticum(Uu)* and *Mycoplasma* species for over 20 years and has done extensive research in this area. Her work has brought these organisms to the attention of maternal-child health practitioners.

The potential of a *Uu* infection of the chorioamnion to cause harm for both the mother and infant demonstrate the need for a closer examination of the relationships between Bacterial Vaginosis (BV), chorioamniotic infection and preterm birth. All women who receive prenatal care at NMCSD and plan to deliver their baby at NMCSD are eligible to participate in the study.

Recent data indicates that:

- U.S. military women may be at increased risk for delivery of preterm births.
- *Ureaplasma urealyticum (Uu)* infection of the chorioamnion is associated with births less than 37 weeks.
- Infection is inversely related to gestational age and birth weight.
- *Uu* infection is a significant cause of pneumonia, meningitis and death in very low birth weight infants.

Major Objectives of the UAB Study are:

1. To determine whether chorioamnion infection , particularly infection with *Uu,* is associated with adverse pregnancy outcome such as premature birth.
2. To identify factors that may predict chorioamnion invasion and preterm birth. Specifically, if the presence of BV is a risk factor for *ureaplasmal* invasion of the chorioamnion.

In order to examine these objectives, women enrolled into the study are screened for *Uu* vaginal colonization and BV at the time of prenatal registration. A group of these women is followed at delivery and recultured for BV and *Uu*. In addition to screening for the presence of BV and *Uu*, the placental tissue and amniotic fluid from the deliveries is cultured for aerobic and anaerobic bacteria and *Trichomonas vaginalis.* Pregnancy outcomes are examined and babies born to these women are assessed for their health status. NP swabs are obtained on all the UAB study babies, The NP swabs are usually obtained in L&D. If a UAB study baby is admitted to the NICU, we will also culture ET aspirate, blood and CSF when available. As a courtesy to the NICU, we also culture specimens from non-UAB study babies. If the doctor orders a specimen for *Uu* and *Mycoplasma,* it is no longer a mail out but is cultured by the UAB lab.

A white 3 ring UAB binder is located in the OB/GYN office. The binder is an available resource to staff. It has the an extensive and frequently updated literature review as well as the condensed version of the research proposal. Thanks for your continued support of the UAB study. Hopefully in the future, we will see results through improved patient outcomes! As always, please feel free to call us with any questions or concerns thay you may have about the study X2-9242.

UAB STUDY

The University of Alabama at Birmingham in collaboration with the Naval Health Research Center and the Naval Medical Center, San Diego, is conducting a study, **"Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcome Among Active-Duty Military Women and Dependent Women."** Researchers were awarded a $1 million dollar grant from DOD to study this issue. The NICU staff will be responsible for laboratory specimens on participating infants. **Specimens are being cultured for *Ureaplasma urealyticum* only.** Therefore, all other cultures should continue to be handled as usual. Participating infants will have a nasal swab completed as soon after delivery as possible. All infants who require spinal taps and/or intubation/ventilation will also have extra CSF and respiratory secretions drawn for culture by the UAB staff. It is important to draw all specimens as soon after delivery as possible and before antibiotics are given. If the staff physician would like blood cultures **for *Ureaplasma urealyticum*** drawn on these infants, the UAB staff can also perform these. In addition, if there is any infant, not necessarily a participating infant, that the staff physician would like to have cultured **for *Ureaplasma urealyticum***, these will also be performed by the UAB staff. A 3 ring binder with the protocol and specific procedures will be at the nursing station for further information. If you have any questions about the study ask Gale or call Paul at 2-9242.

UAB STUDY UPDATE

As most of you know, the UAB Study is well under way. We have already enrolled 1,650 women over the last 16 months with 375 deliveries. For those of you who are new to the NICU or if you would just like a refresher, this update may help answer some questions that you may have about the study.

The University of Alabama at Birmingham (UAB) in collaboration with the Naval Health Research Center (NHRC) and the Naval Medical Center, San Diego (NMCSD), is conducting a study, **"Risk Factors for Chorioamnion Infection and Adverse Pregnancy Outcomes Among Active-Duty Military and Dependent Women."** Dr. Gail Cassell, Professor and Chairman of the Department of Microbiology at UAB was awarded a grant from the Department of Defense to study this issue. Dr. Cassell has been studying *Ureaplasma urealyticum(Uu)* and *Mycoplasma* species for over 20 years and has done extensive research in this area. Her work has brought these organisms to the attention of maternal-child health practitioners.

The potential of a *Uu* infection of the chorioamnion to cause harm for both the mother and infant demonstrate the need for a closer examination of the relationships between Bacterial Vaginosis (BV), chorioamniotic infection and preterm birth. All women who receive prenatal care at NMCSD and plan to deliver their baby at NMCSD are eligible to participate in the study.

Recent data indicates that:

- U.S. military women may be at increased risk for delivery of preterm births.
- *Ureaplasma urealyticum (Uu)* infection of the chorioamnion is associated with births less than 37 weeks.
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Major Objectives of the UAB Study are:

1. To determine whether chorioamnion infection , particularly infection with *Uu,* is associated with adverse pregnancy outcome such as premature birth.
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In order to examine these objectives, women enrolled into the study are screened for *Uu* vaginal colonization and BV at the time of prenatal registration. A group of these women is followed at delivery and recultured for BV and *Uu*. In addition to screening for the presence of BV and *Uu*, the placental tissue and amniotic fluid from the deliveries is cultured for aerobic and anaerobic bacteria and *Trichomanas vaginalis.* Pregnancy outcomes are examined and babies born to these women are assessed for their health status.

That's where the NICU comes in. NP swabs are obtained on all the UAB study babies, The NP swabs are usually obtained in L&D. If a UAB study baby is admitted to the NICU, we will also culture ET aspirate, blood and CSF when available.

Media for UAB specimens are in the small lab refrigerator in the unit. The small clear tubes have a yellow "fluid" media and require being warmed to room temperature before introducing the specimen. 0.2 - 0.5 cc of blood and CSF are adequate to inoculate the media. NP and tracheal aspirate specimens may be introduced into the media with dacron tipped swabs (these are also found in the lab refrigerator in a clear plastic bag marked UAB). Tracheal aspirate may be flushed with sterile normal saline to get it through the suction catheter. Once inoculated, the media tubes need to be labeled with the name of patient , type of specimen and date. Then the tube needs to be put back into the UAB study rack in the lab refrigerator and UAB study staff needs to be notified that the specimen was obtained. A message that a specimen was

obtained can be left at 2-9242 or page Mara at 979-3609, and the specimen will be retrieved. If antibiotics have already been started or if the blood is drawn from a site that was not properly prepped, don't panic. The specimen is still acceptable.

As a courtesy to the NICU, we also culture specimens from non-UAB study babies. If the doctor orders a specimen for *Uu* and *Mycoplasma*, it is no longer a mail out but is cultured by the UAB lab. Label it with name, source and date placing it in the refrigerator and page UAB study staff. If you have any questions or concerns please call our office at 2-9242 or page Mara Berzins at 979-3609 or the on-call Microbiologist at 979-2290.

A white 3 ring UAB binder is located at the NICU front desk. The binder is an available resource to the NICU staff. It has the NICU protocol and procedures as well as an extensive literature review and a condensed version of the research proposal.

Thanks for your help with the UAB study hopefully we will see results through improved patient outcomes!

UAB STUDY

CHORIOAMNION INFECTION STUDY

RESEARCH PROPOSAL, PROCEDURES AND REVIEW OF THE LITERATURE

X9242

979-3609/979-2087

UAB STUDY

INFORMATION

Beginning today, February 24, 1997, for participating women who deliver vaginally we will be collecting placenta, cord blood, nasal swabs, only on a random sample of women. We will however, need you to continue swabbing all UAB participants upon admission, as well as collecting maternal serum during their routine lab draw at admission. We must collect swabs and maternal serum on all participating women on admission because we are uncertain of who will ultimately deliver by c/s. We will continue to collect all specimens from all c/s also. C/S intact membranes, are our study "gods", and, need the most diligent care in proper collection of all specimens. This does not mean that ruptured membrane c/s or the random sample of vaginal deliveries are less important. These participants also need diligent collection of specimens to ensure a good study outcome. We GREATLY appreciate all the efforts you have all taken to ensure the success of this study. Please continue to let us know if you are having any difficulties of any kind or if we can do anything to help you or make this easier for you.

There is a new checklist in the packet. Please continue to use this checklist as a reminder of what needs to be collected and how to properly reach UAB study staff.

THANK YOU,

Gale, Paul, Debby, Julie, and Jackie ☺

DATE: 7 April 1997

FROM: UAB Study Staff
TO: L & D Nursing Staff

SUBJECT: UAB Chorioamnion Infection Study Update

A big **THANKS** for all the extra effort put forth for the UAB Study! Just a couple of reminders to keep the study on track:

It is *very important* to initiate the **L & D checklist** and to write UAB on the board for *every* UAB Study participant that is admitted to L&D. Also, placing a blue UAB study sticker on the hard maroon chart makes it much easier for us to identify the patient as a study participant.

It is crucial that *all* UAB study participants have the initial vaginal swabs and blood drawn upon being admitted. If a woman is randomly selected by the UAB to complete the study, we will make sure the nurse caring for that patient and the charge nurse are aware that the patient has been "randomly selected" and will need *all* specimens collected.

For *all* women delivering by csec, it is very important to complete the study and collect all specimens, these include: the placenta, cord blood, amniotic fluid and infant's nasal swabs as well as the vaginal swabs and maternal blood that are collected on admission (the L&D checklist is a good reference to help one remember which specimens have/have not been collected). Please put the placenta in the sterile zip-lock bags provided in the UAB packet and keep the placenta at room temperature. If the placenta needs to be sent to pathology, the UAB study staff will do this after the study criteria have been completed. Be sure a pathology CHIT has been completed and attached to the placenta. Please do not put the UAB placenta in Formalin. The Formalin will kill any bacteria present.

When a UAB study participant has had a pre-op workup for a scheduled c-section, please notify us, so that we can prepare the for the placental work-up. The workup of the placenta takes about two hours so any preparation done ahead of time really helps.

The UAB study packets are located in the bottom drawer of the file cabinet in the prep room and the bags with the specimen tubes are in the prep room refrigerator. UAB study staff are always available for any questions or problems you may have. To reach us call 2-9242 or from 0500-1700 pager 979-8440; and from 1700-0500 Monday -Friday and Saturday and Sunday all day call 979-2290.

Currently we have enrolled over 818 woman into our study. Of these woman, approximately 60% are colonized by Ureaplasma urealyticum and about 20% of our population has Bacterial Vaginosis. What does this mean????? Also, several of the premature infants in the NICU have been colonized with Ureaplasma urealyticum.

Thanks for the great effort that you are putting into the UAB study! Hopefully, all the effort will be rewarded with improved patient outcomes (both maternal and neonatal) in the future! FYI -Attached you will find a copy of the L&D checklist and a summary of the UAB Study.

UAB STUDY
UPDATE

TO THE L&D STAFF:

KEEP UP THE GREAT WORK IDENTIFYING STUDY PARTICIPANTS AND OBTAINING THE SWABS AND BLOOD!! JUST A FEW TIDBITS TO MAKE YOUR LIFE (AND OURS) EASIER....

THANKS TO MAUREEN'S GREAT SUGGESTIONS, WE WILL BEGIN PLACING BRIGHT YELLOW DOTS ON THE BLUE CARDS OF UAB STUDY PARTICIPANTS WHEN THEY ENROLL. IT WILL BE 7-8 MONTHS BEFORE YOU ROUTINELY SEE YELLOW DOTS ON ALL STUDY PARTICIPANT'S BLUE CARDS. THERE ARE YELLOW DOTS IN THE PREP ROOM. IF YOU ARE WORKING IN THE PREP ROOM, FEEL FREE TO PUT THE STICKERS ON UAB STUDY PARTICIPANT'S BLUE CARDS (WHO ARE NOT ADMITTED) TO MAKE THEM EASIER TO IDENTIFY WHEN THEY RETURN.

YELLOW REMINDER CARDS WILL BE PLACED ON THE SHARPS BOXES IN EACH BIRTHING ROOM AS A REMINDER TO OBTAIN UAB BLOOD WHEN DRAWING LABS ON PATIENTS.

ALL UAB STUDY WOMEN NEED INITIAL VAGINAL SWABS AND BLOOD DRAWN UPON ADMISSION. WE ARE STILL COMPLETING THE STUDY ON ALL C-SECTIONS. HOWEVER, THE ONLY VAGINAL DELIVERIES COMPLETED ARE THOSE THAT ARE RANDOMLY SELECTED. A LAMINATED YELLOW CARD STATING "THIS WOMEN HAS BEEN RANDOMLY SELECTED TO COMPLETE THE STUDY" WILL BE PLACED ON THE BOARD AND THE PATIENT'S NURSE AND THE CHARGE NURSE WILL BE NOTIFIED.

LASTLY, ON THE COMPUTER ADMIT RECORD, THERE IS A SPACE ASKING IF THE WOMAN IS PARTICIPATING IN ANY STUDIES. THIS A GOOD WAY TO CHECK YOUR PATIENT'S UAB STUDY STATUS. (THANK YOU MELISSA!!)

AS ALWAYS, PLEASE FEEL FREE TO CALL IF YOU HAVE ANY QUESTIONS
X2-9242 OR 979-2087, 979-3609

UAB STUDY UPDATE

It is hard to believe that over **1500** women have been enrolled into the UAB Study. So far we have had 346 deliveries, 67 SAB's and 11 IUFD's. Thanks to all of your hard work, 128 of 346 UAB placentas have been studied so far. To ensure the *most* accurate results, we have included a few important points to remember when collecting samples:

- Please remember to allow the small media tubes to warm to room temperature before inoculating them with the vaginal swabs (the cold media could kill the Ureaplasma and other bacteria).

- Be sure to use only 1 swab per media tube and 1 swab for the slide (5 swabs in all) if only 1 swab is used for all tubes or for more than 1 tube, a dilutional effect may occur. Extra swabs can be found in the bottom drawer of the prep room file cabinet along side the UAB packets.

- Refrigerate the media tubes immediately after inoculating them to ensure that the Ureaplamsa are held viable, if they are left out at room temperature they may die.

- Be sure the blue UAB baby sheet goes on the babies chart, even if the baby is fine. Occasionally, a study baby may be admitted from 3 west to the NICU.

Hopefully, the yellow selection card is helping to identify vaginal deliveries selected to complete the study. The yellow card is placed on the board to identify women who have been randomly selected to complete the study if they have vaginal deliveries. But don't forget, **all** UAB study women who deliver by c-sec complete the study.

Yellow reminder cards have also been placed in all of the birthing rooms and prep room, as a reminder to obtain blood and swabs on all study women.

To help Prep room staff identify study participants, women enrolling in the study will have a yellow "dot" placed on their blue outpatient cards. The yellow "dot" along with the big blue UAB sticker on their outpatient charts should make identifying UAB study participants easier. Remember the lag time between enrollment into the study and delivery date means yellow dots will not be appear on the blue cards for a few more months. If a study participant comes to the Prep Room and does not have a yellow sticker on her blue card there are extra yellow stickers on the board in the prep room to place on the participants blue outpatient card. But remember, this yellow sticker my not always appear on her blue outpatient card.

To help identify UAB babies, a yellow "dot" will be placed on the blue infant nursing sheet when the women is admitted to L&D. This should make tracking the babies easier too.

Your help and commitment to the UAB Study is greatly appreciated and a big part in the success of the study. As always feel free to call us if you have any problem, questions, gripes or even words of encouragement. **Thank you, thank you, thank you!!!!!**

Mara, Paul, Julie, Tom and Jackie X2-9242

UAB STUDY L&D CHECKLIST

At Admission:

_____ 1. Pull plastic media bag with slide from refrigerator to warm to room temperature.
_____ 2. Draw 1 extra marble top with routine labs (do not send to lab/place in plastic media bag with stamper label and mark **"MATERNAL"** on label).
_____ 3. On 1st vaginal exam (**prior to KY jelly use**) swab vaginal vault for secretions with 4 dacron swabs (**NOT CERVIX**).
_____ 4. Smear slide with 1 vaginal swab prior to inoculating media with same swab (write study #, last 4 of SSN, & date on white part of slide with **pencil only**). Return smeared slide to plastic media bag in front "pocket".
_____ 5. Inoculate each media "bullet" with single swab by swishing swab in media, wring out swab on top inner portion of media bullet and **dispose of swab (DO NOT LEAVE SWAB INSIDE MEDIA)**. Replace cap on media bullet **securely**. Return inoculated media to plastic bag and place bag back in lab fridge with stamper label on outside of bag. Retain **NASAL** "bullet" at patient's bedside for use with infant's nasal swab after delivery.
_____ 6. Place blue "UAB STUDY" sticker on mom's L/D chart.
_____ 7. Page UAB Study staff when delivery is **imminent** (within 1 hour or so of delivery) to pick up specimens.

M-F/0500-1700/**979-2087** **M-F**/1700-0500/**979-2290**
S/S (0500 Saturday through 0500 Monday) **979-2290**

At Delivery:

_____ 1. 5 to 10 cc amniotic fluid for **C/S ONLY** (place in sterile syringe and recap. Place with placenta with stamper label on).
_____ 2. 5 to 10 cc cord blood (Place in marble top with stamper label and mark **"CORD"** on label. Place with placenta.).
_____ 3. Placenta (place in 2 sterile bags via sterile technique/label with stamper label). **Do not send placenta to pathology.** UAB study personnel will retrieve placenta from patient's room upon arrival to L&D. Please keep placenta at room temperature, **unless otherwise specified by UAB study personnel**. UAB Study will complete study criteria and take placenta to pathology after. Please have CHIT ready for pathology when UAB Study picks up specimens.

After Delivery (INFANT):

_____ 1. Nasal Swab (aluminum shaft Dacron Swab)
_____ 2. Inoculate room temperature media "bullet" with single swab by swishing swab in media, wring out swab on top inner portion of media bullet and **dispose of swab (DO NOT LEAVE SWAB INSIDE MEDIA)**. Replace cap on media bullet **securely**. Place with placenta for pickup by UAB staff with stamper label on bullet.
_____ 3. Place blue "UAB STUDY" paper in baby's chart.

Return all unused items and checklist to plastic bag to be retrieved with specimens.

I AM A UAB STUDY INFANT

If I get sick and need an L/P or am placed on a ventilator please follow the UAB Study protocol for me.

Thank You ☺

If you have any questions, you can call the UAB Study office at 2-9242, during normal working hours, or page Paul Stamper at anytime, day or night, at 979-2087.

The UAB Study Thanks You!

We've reached 1,000 enrollees, thanks to your efforts! Keep up the good work.

The UAB Study Staff

UAB STUDY
RECYCLE BOX:

Unused Nasal tubes, checklists, sterile placenta bags, UAB stickers, swabs, bags and any other clean and unused UAB "stuff"

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